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**CLINICAL DETERIORATION DURING
ANTITUBERCULOSIS TREATMENT IN A
HIGH HIV-1 PREVALENCE SETTING**

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DOCTOR OF MEDICINE

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Dedication

To my parents, Winston and Cheryl, I hope the dedication of this work to you will, in some small way, thank you for your never-ending love and guidance.

To my beautiful and beloved wife, Feriyi, I look forward with great anticipation to the life we will share together. I celebrate this achievement with you.

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Abstract

Clinical Deterioration during Antituberculosis Treatment in a High HIV-1 Prevalence Setting

Dominique Justin Pepper

Background

Tuberculosis and HIV-1 pose a number of clinical challenges, one of which is clinical deterioration during antituberculosis treatment. The foci of this thesis were two-fold. The first objective was to determine the incidence, causes, risk factors and outcomes of clinical deterioration in a high HIV-1 prevalence setting. The second was to describe the rare but potentially lethal condition of neurologic tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS).

Methods

Three studies were conducted; two at a referral hospital and one at a tuberculosis clinic in Cape Town, South Africa. The first study was conducted at GF Jooste Hospital over a 3-month period to determine the causes for clinical deterioration. We subsequently conducted a 9-month prospective study at Site B Khayelitsha tuberculosis clinic to determine the incidence, causes, risk factors and outcomes of clinical deterioration. Lastly, we retrospectively reviewed patients presenting with neurologic TB-IRIS to GF Jooste Hospital over a 29 month period.

Significant Findings and Conclusions

Our first study found that 17% of medical inpatient admissions at GF Jooste Hospital were due to clinical deterioration during antituberculosis treatment. Drug resistant *Mycobacterium tuberculosis* and bacterial infections were important reasons for clinical deterioration and death. Additional illnesses to tuberculosis accounted for most referrals, particularly new AIDS-defining illnesses and bacterial infections. In the subsequent study at the tuberculosis clinic, we found that 40% of patients starting antituberculosis treatment experienced clinical deterioration. Frequent causes for clinical deterioration in this setting were co-morbid illnesses, tuberculosis-related illnesses and AIDS- defining illnesses (in decreasing frequency). Significant risk factors for clinical deterioration were HIV-1 infection and profound immune suppression at tuberculosis diagnosis. The clinical outcomes after 24 weeks of antituberculosis treatment were concerning: 22% of patients were lost to follow-up and 8% of patients died. Lastly, we report the first case series of neurologic TB-IRIS. In a hospital setting, 10% of patients diagnosed with TB-IRIS had neurologic involvement. Outcome was fair; 13% of patients with neurologic TB-IRIS died and 87% required hospital admission (median of 12 days). In summary, clinical deterioration during antituberculosis treatment is a formidable challenge in our high HIV-1 prevalence setting. Novel strategies are urgently needed to address this high burden of disease.

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Supervisors

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Declaration

I, Dominique Justin Pepper, do hereby declare that the research presented in this thesis was conceived and executed by myself, except where otherwise indicated.

Neither the substance nor any part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in the University or any other University. This thesis is presented in fulfilment of the requirements for the degree of MD.

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Publications arising from work related to this thesis

Pepper DJ, Marais S, Wilkinson RJ, Bhaijee F, Maartens G, McIlleron H, De Azevedo V, Cox H, McDermid C, Sokhela S, Patel J, Meintjes G. Clinical Deterioration during Antituberculosis Treatment in Africa: Incidence, Causes and Risk Factors. *BMC Infectious Diseases*. **2010**; 10:83

Pepper DJ, Marais S, Maartens G, Morroni C, Rebe K, Rangaka MX, Oni T, Wilkinson RJ, Meintjes G. Neurological Manifestations of Paradoxical Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome: A Case Series. *Clinical Infectious Diseases*. **2009**; 48 (11):e96-107

Pepper DJ, Rebe K, Morroni C, Wilkinson RJ, Meintjes G. Clinical deterioration during antituberculosis treatment at a district hospital in South Africa: the importance of drug resistance and AIDS defining illnesses. *PloSONE* **2009**; 4(2):e4520

Pepper DJ, Meintjes G, McIlleron H, Wilkinson RJ. Combined therapy for tuberculosis and HIV-1: the challenge for drug discovery. *Drug Discovery Today*. **2007**; 12 (21-22):980-989

Marais S, Scholtz P, **Pepper DJ**, Meintjes G, Wilkinson RJ, Candy S. Neuroradiological features of the tuberculosis-associated immune reconstitution inflammatory syndrome. *International Journal of Tuberculosis and Lung Disease*. **2010**;14(2):188-96.

Marais S, Wilkinson RJ, **Pepper DJ**, Meintjes G. Management of patients with the immune reconstitution inflammatory syndrome. *Current HIV/AIDS Reports*. 2009; 6(3):162-71

Oni T, **Pepper DJ**, Wilkinson RJ. HIV-TB Drug Interactions chapter in *AIDS and Tuberculosis: A Deadly Liaison*. **2009**; Infection Biology Handbook Series. Wiley Blackwell. pg. 123-153.

Bhaijee F, Wainwright H, Meintjes G, Wilkinson RJ, Todd G, de Vries E, **Pepper DJ**. Warfarin Induced Skin Necrosis in HIV-1 Infected Patients with Tuberculosis and Venous Thrombosis: a case series. **2010**; *South African Medical Journal*. [in press]

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Chapter 1

Background to the Thesis and Scope

University of Cape Town

Contributions: The candidate compiled the initial draft and critically revised the chapter and figures 1.1 and 1.2. Robert J. Wilkinson and Graeme Meintjes critically revised the chapter and figures 1.1 and 1.2.

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Tuberculosis and HIV-1 cause considerable morbidity and mortality. One-third of the world's population is infected with *Mycobacterium tuberculosis* (*M.tb*). Almost nine million new cases of tuberculosis and approximately two million tuberculosis deaths occur annually (Corbett 2003, WHO 2006a). In 2008, almost 33.4 million people were infected with HIV-1 and 2.0 million people died of the Acquired Immune Deficiency Syndrome (AIDS) (UNAIDS 2009).

Co-infection is an increasingly common problem. In 2000, almost 11 million people were co-infected with HIV-1 and tuberculosis (Corbett 2003), the majority of whom were in the developing world (Dye 1999). Sub-Saharan Africa epitomises the synergy of these two pandemics. More than half of tuberculosis patients tested are HIV-1 infected (WHO 2007) and many of the 22.5 million HIV-1 infected people are co-infected with *M.tb* (WHO 2005). The annual incidence of tuberculosis doubles within the first year of HIV-1 infection (Sonnenberg 2005) and may exceed 30% per annum in the profoundly immune-suppressed (Wood 2000). Likewise, tuberculosis disease accelerates the progression of HIV-1 infection to AIDS by inciting viral replication in immunologically activated CD4⁺ cells (Collins 2002, Lawn 2001, Toosi 2001).

Often dual antituberculosis and antiretroviral treatment is necessary to save co-infected patients. Chapter 2 describes the challenges of treating these patients. Few studies in high HIV-1 prevalence settings have prospectively investigated the entity of clinical deterioration during antituberculosis treatment. Chapter 3 describes our first study at GF Jooste Hospital, in which

we prospectively investigated the causes and frequency of clinical deterioration over a 3-month period.

Using experience gained from the first study, advice from senior colleagues and reports from the published literature, I formulated an algorithm to systematically determine the reasons for clinical deterioration (Figures 1.1 and 1.2). The accompanying poster was placed in the Accident and Emergency Unit, where it received favourable reviews from doctors in the departments of Internal Medicine and Emergency Medicine. Possible reasons for clinical deterioration include: i) poor adherence (Brudney 1992), ii) drug toxicities from antimicrobial treatment (Hoffman 2007, Kwara 2005, McIlleron 2007, Pepper 2007) iii) drug resistant *M.tb* (Small 1993, Gandhi 2006), iv) co-morbid illnesses (Ansari 2002, Greenberg 2005, Eza 2006, Martinson 2007), v) an alternate illness to tuberculosis, vi) tuberculosis associated-immune reconstitution inflammatory syndrome (TB-IRIS) (Narita 1998, Lawn 2005), vii) a paradoxical tuberculosis reaction (if not receiving ART), viii) incorrect antituberculosis treatment, and ix) malabsorption of antimicrobial treatment (McIlleron 2006).



Figure 1.1 An Approach to Deterioration during TB Treatment

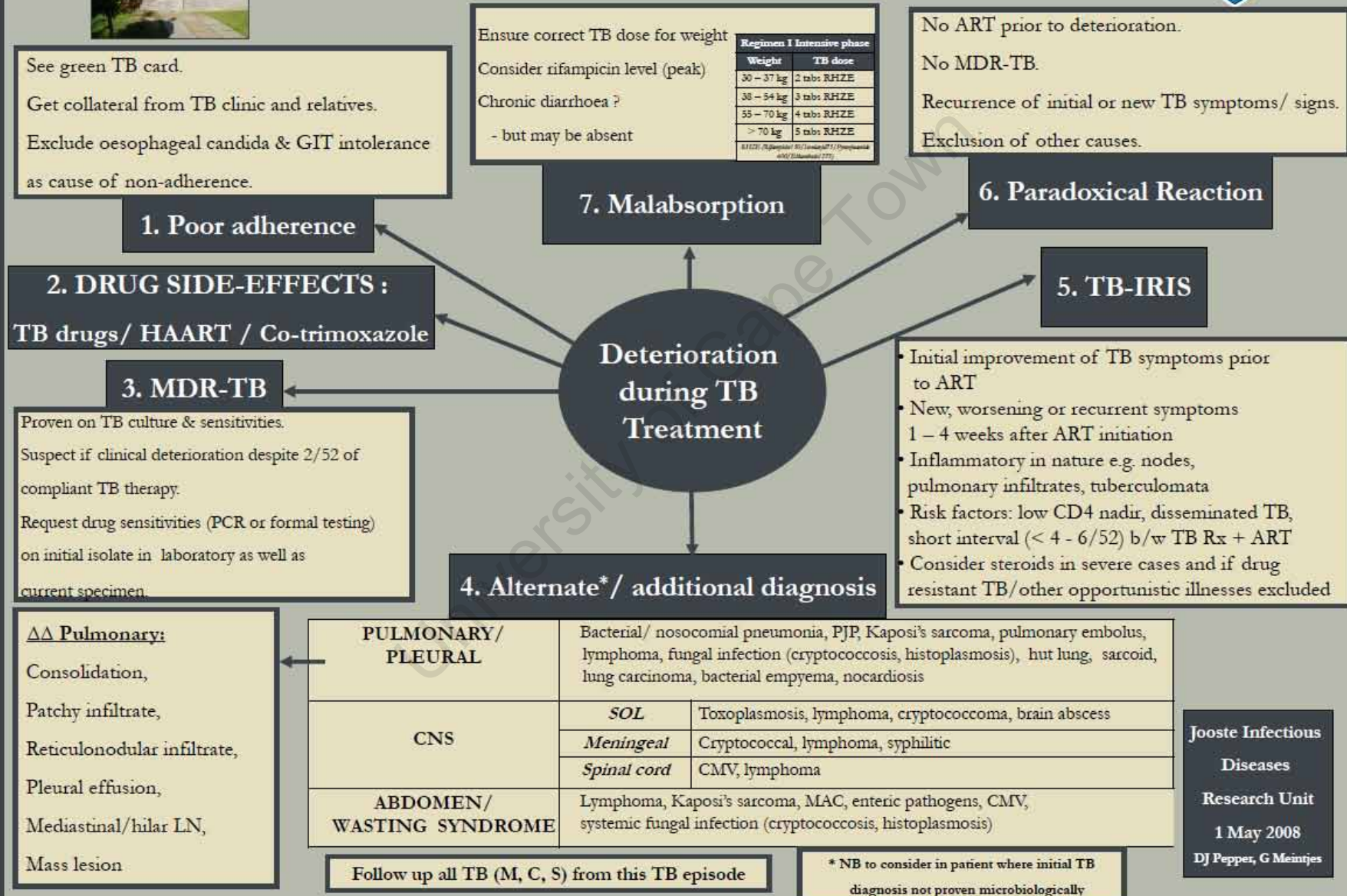


Figure 1.2a: Deterioration during TB treatment – History checklist

Presenting complaint

- List each presenting complaint and its duration

History of this TB episode

- Initial TB symptoms and likely site of disease (e.g. pulmonary, pleural, meningeal)
- TB diagnosis
 - Final smear, culture and sensitivity results sent at initial diagnosis
 - If smear and culture negative: reason for empiric diagnosis (e.g. USS, LP results)
- Date started TB treatment
- Current TB regimen
- Response to TB treatment (if any): symptoms and weight

HIV/ ART history

- HIV status not known: offer VCT
- If HIV infected
 - Date of HIV diagnosis
 - WHO Stage, Baseline CD4 and date
 - ART initiation date, regimen and doses
 - Results of latest CD4 and VL if > 6 months ART
- If HIV uninfected
 - Date last test performed, if >6 months offer VCT
 - Is there another reason for immune suppression?

Past medical history

- Previous TB (details)
- Other opportunistic infections (details)
- Other medical illnesses
- Recent hospital admission (<60 days)

Medication

- List all medication patient is receiving as well as the dosage
- Traditional medication/ over the counter medication? Drug allergies?

Assess adherence

- HAART (how many doses missed during preceding 3 days?)
- TB treatment (?DOTS, check clinic card)

Social history

- Alcohol, smoking, drugs (previous/ current, quantify)

Figure1.2b: Diagnostic Algorithm for Clinical Deterioration during Antituberculosis Treatment

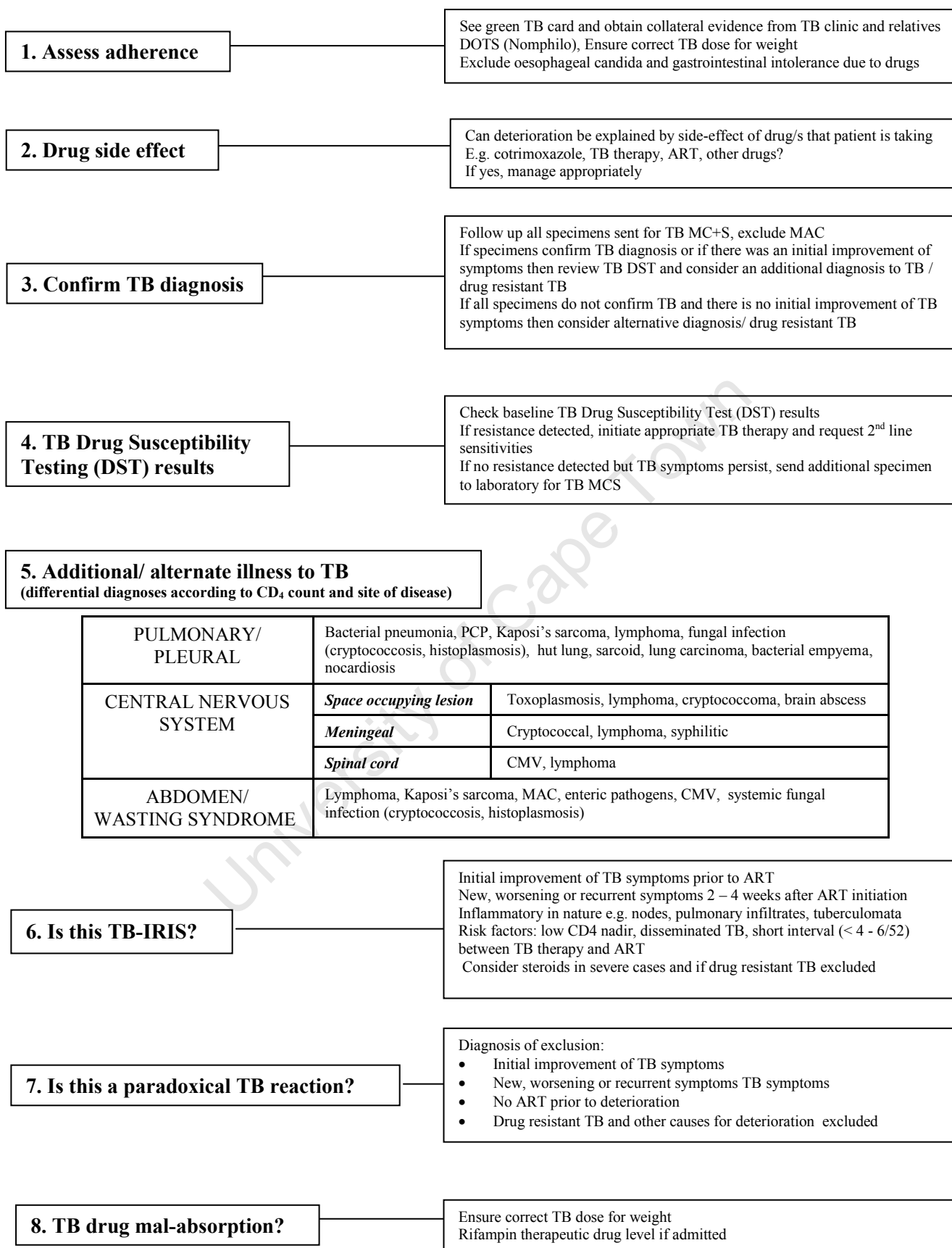


Figure 1.2c: Relevant Investigations and Management prior to Consultant Review

PULMONARY/ PLEURAL	Bacterial pneumonia, PCP, Kaposi's sarcoma, lymphoma, sarcoid, fungal (cryptococcosis, histoplasmosis), lung carcinoma, bronchiectasis, bacterial infection, empyema, nocardiosis
<ol style="list-style-type: none"> 1. Sputum TB microscopy and culture if cough or infiltrate 2. Induced sputum if non-productive cough 3. Sputum MCS 4. CXR (consider lateral decubitus CXR if suspect aspergilloma or to demonstrate pleural fluid run-off) 5. Examine skin and oral mucosa for Kaposi's sarcoma if reticulonodular infiltrate +/- nodes 6. Oxygen saturation (finger probe), check for exertional desaturation if PCP suspected 7. Arterial blood gas if respiratory rate >30 or sats <95% room air 8. Bacterial blood culture prior to broad spectrum antibiotics 9. Consider nosocomial sepsis if > 48hrs admission or admitted to hospital within preceding 60 days 10. Pleural tap if pleural effusion to exclude empyema, send for WCC+diff, ADA, TB MCS +/- cytology and bacterial MCS 	

CENTRAL NERVOUS SYSTEM	<i>Space occupying lesion</i>	Toxoplasmosis, lymphoma, cryptococcoma, brain abscess
	<i>Meningeal</i>	Cryptococcal, lymphoma, syphilitic, bacterial
	<i>Spinal cord</i>	CMV, lymphoma
	<i>Encephalitis/ encephalopathy</i>	HIV, drugs such as INH and efavirenz
<ol style="list-style-type: none"> 1. Blood sugar and serum sodium for all confused/ fitting patients 2. If no focal neurology/ papilloedema/ cerebellar signs: <ul style="list-style-type: none"> • 10ml lumbar puncture (opening pressure/ chemistry/ cells/ India-Ink/ CLAT/ cryptococcal culture/ TB MCS/ syphilis serology – RPR and FTA-Abs) 3. If lumbar puncture contraindicated: <ul style="list-style-type: none"> • Serum CLAT • Toxoplasmosis and syphilis serology – RPR and VDRL • Aseptic blood culture prior to Ceftriaxone • CT brain, if no contraindication then lumbar puncture 		

ABDOMEN/ WASTING SYNDROME	Adverse drug reaction, lymphoma, <i>Non-salmonella typhi</i> , Kaposi's sarcoma, MAC, UTI, enteric pathogens (esp. if diarrhea or vomiting), CMV, systemic fungal infection (cryptococcosis, histoplasmosis), HIV wasting
<ol style="list-style-type: none"> 1. If abdominal pain: <ul style="list-style-type: none"> • ALT, ALP, lipase, lactate (if > 6months D4T/DDI) • If transaminitis (ALT > 160) stop all hepatotoxic drugs, check HepBsAg, INR, total bilirubin 2. Urine: <ul style="list-style-type: none"> • Dipstix if jaundice/ dysuria/ haematuria, pregnancy test in all women • MCS if urinary symptoms 3. Stool: <ul style="list-style-type: none"> • Coccidian parasites (<i>Cryptosporidium</i>, <i>Microsporidium</i>, <i>Isospora belli</i>) if chronic (> 2 weeks) • Stool MCS if inflammatory (pus, blood), also blood culture 4. Consider abdominal USS 5. Aseptic ascitic tap if ascites 6. Appropriate antibiotics if clinically septic or acute inflammatory diarrhea 7. MycoFlytic blood culture if MAC or fungal infection considered 8. Skin biopsy if appropriate 	

In order to determine the burden of clinical deterioration in our high HIV-1 prevalence setting, I used this algorithm during a prospective observational study at a tuberculosis clinic (Site B Khayelitsha, Figure 1.3). Chapters 4-5 describe the incidence, causes, risk factors and outcomes of clinical deterioration in this high HIV-1 prevalence setting. This work will be important in defining future priorities for tuberculosis and ART programmes in South Africa.

TB-IRIS is a unique form of clinical deterioration that occurs in profoundly immune-suppressed patients who receive both antituberculosis and antiretroviral treatment. Chapter 6 describes the clinical manifestations and outcomes of neurologic TB-IRIS, which is the most severe manifestation of TB-IRIS. This is the first reported case series of neurologic TB-IRIS.

Finally, chapter 7 discusses strategies that may alleviate the high burden of clinical deterioration. Chapter 7 also discusses future work arising from this thesis.

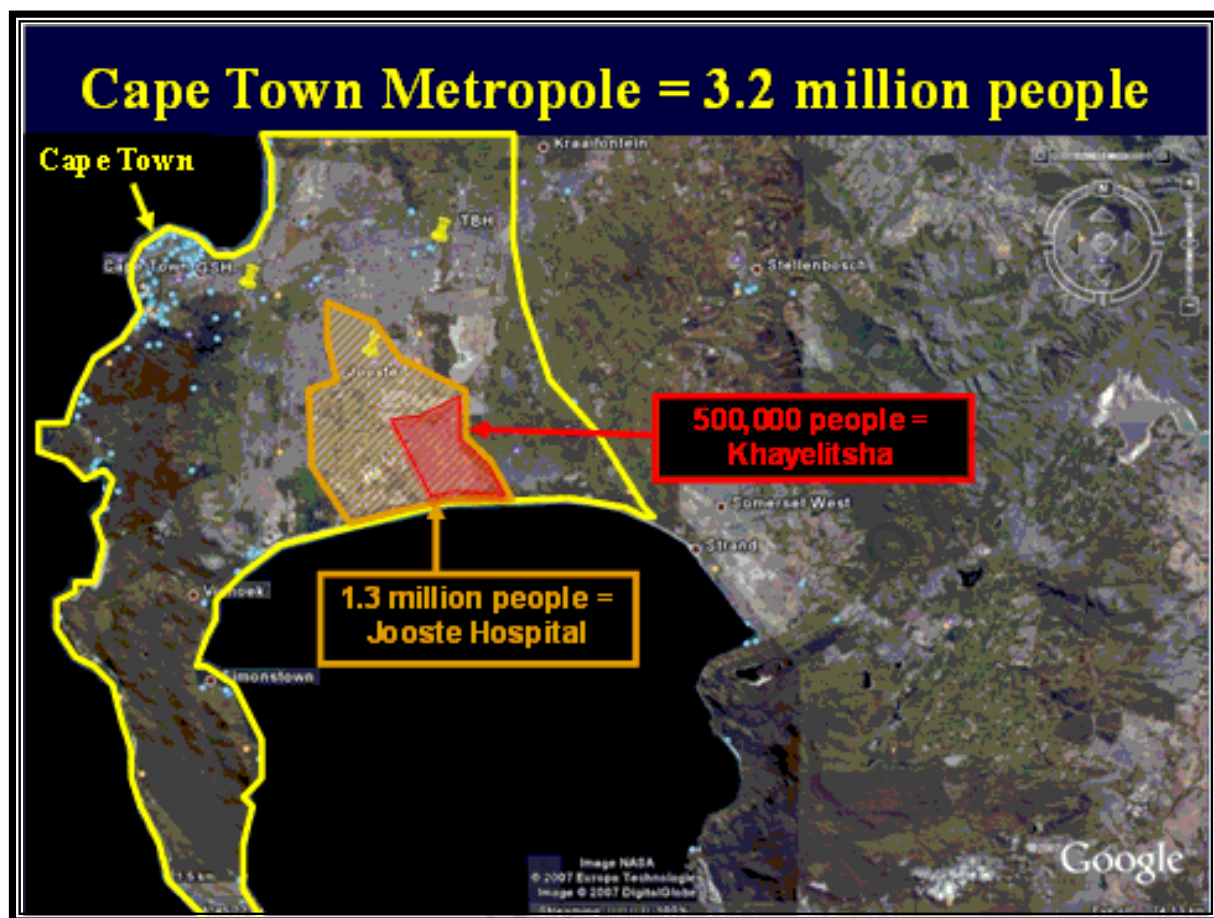


Figure 1.3 Map of Cape Town Metropole (bordered yellow) showing the catchment population of GF Jooste Hospital (bordered orange) and the township of Khayelitsha (bordered red)

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Chapter 2

The challenges of tuberculosis and HIV-1

University of Cape Town

Contributions: The candidate compiled the initial draft and critically revised this chapter.

Figure 2.1, and tables 2.2, 2.3 and 2.4 are obtained from a review paper by Oni *et al.* The figure and tables were conceived, designed and completed by the candidate. Robert J. Wilkinson, Graeme Meintjes, Gary Maartens, Helen McIlleron, Tolullah Oni and Feriyl Bhaijee critically revised portions of this chapter.

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1. **Pepper DJ**, Marais S, Wilkinson RJ, Bhaijee F, Maartens G, McIlleron H, De Azevedo V, Cox H, McDermid C, Sokhela S, Patel J, Meintjes G. Clinical Deterioration during Antituberculosis Treatment in Africa: Incidence, Causes and Risk Factors. *BMC Infectious Diseases*. **2010**; 10:83
2. **Pepper DJ**, Marais S, Maartens G, Morroni C, Rebe K, Rangaka MX, Oni T, Wilkinson RJ, Meintjes G. Neurological Manifestations of Paradoxical Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome: A Case Series. *Clinical Infectious Diseases*. **2009**; 48 (11):e96-107
3. **Pepper DJ**, Rebe K, Morroni C, Wilkinson RJ, Meintjes G. Clinical deterioration during antituberculosis treatment at a district hospital in South Africa: the importance of drug resistance and AIDS defining illnesses. *PloSONE* **2009**; 4(2):e4520
4. **Pepper DJ**, Meintjes G, McIlleron H, Wilkinson RJ. Combined therapy for tuberculosis and HIV-1: the challenge for drug discovery. *Drug Discovery Today*. **2007**; 12 (21-22):980-989
5. Oni T, **Pepper DJ**, Wilkinson RJ. HIV-TB Drug Interactions chapter in *AIDS and Tuberculosis: A Deadly Liaison*. **2009**; Infection Biology Handbook Series. Wiley Blackwell. pg. 123-153.

Tuberculosis is the commonest cause of morbidity and mortality in HIV-1 infected people in sub-Saharan Africa (Corbett 2003). The mortality associated with tuberculosis is considerably higher in HIV-1 infected than HIV-1 uninfected patients (Ackah 1995). Two reasons for the

higher mortality are: i) the rapid progression of tuberculosis in HIV infected people (Corbett 2004), and ii) the delay in diagnosis due to the reduced sensitivity of sputum smear and the atypical manifestations of disease (Harries 2001, Mendelson 2007). Post-mortem studies of hospitalized HIV-1 infected patients report a myriad of diseases causing deterioration and death. Unfortunately, most diagnoses considered of clinical significance are not suspected ante-mortem (Eza 2006, Borges 1997). A post-mortem study from Botswana reported that among HIV-1 infected patients, the most common pathologic findings were tuberculosis (40%), bacterial pneumonia (23%), *Pneumocystis jirovecii* pneumonia (11%), and Kaposi's sarcoma (11%); these conditions were the cause of death in 38%, 14%, 11%, and 6%, respectively (Ansari 2002). Similarly, Martinson *et al* from South Africa found that concomitant disseminated tuberculosis and severe bacterial infections, including salmonellosis, were the leading co-morbid illnesses in 47 patients who died with advanced HIV-1 disease.

Timely antituberculosis, antiretroviral and other antimicrobial treatment should prevent these unnecessary deaths. Numerous factors are responsible for delays in the diagnosis and treatment of tuberculosis (Storla 2008), one of which is the delay of microbiologic confirmation of tuberculosis. In the South African public sector, rapid tuberculosis diagnostics, such as the GenoType® MTBDRplus (*Hain Lifescience*), are not consistently used in all centres. Often, antituberculosis treatment is empirically commenced using WHO case definitions for smear negative pulmonary and extra-pulmonary tuberculosis (WHO 2006b). This likely facilitates earlier initiation of antituberculosis treatment and prevents deterioration due to progressive tuberculosis. Over the past few years, there was considerable debate regarding the optimal interval to initiate ART after tuberculosis diagnosis. Recently, a trial from KwaZulu Natal showed benefit when commencing ART during antituberculosis treatment (Karim 2009). The

following paragraphs describe the antituberculosis and antiretroviral treatment regimens in South Africa during the course of my research.

2.1 Rationale of ART & antituberculosis treatment in the South African public sector

Antituberculosis treatment is a multi-drug regimen given over a long period of time. Single agent antituberculosis treatment rapidly gives rise to drug-resistant organisms (Mitchison 2005). Possible explanations include: *M.tb* divides slowly, it is metabolically capable of becoming drug insensitive and/or bacilli may become sequestered (Connolly 2007). The advent of rifampin and pyrazinamide allowed highly effective ‘short course’ antituberculosis regimes. In South Africa (SATB 2004), the national tuberculosis programme manages new tuberculosis cases with 6 months of treatment (isoniazid, rifampin, pyrazinamide, and ethambutol [HRZE] for 2 months followed by HR for 4 months [2HRZE/4HR]). The retreatment regimen adds streptomycin (S) as follows 2HRZES/1HRZE/5HRE. New tuberculosis cases do not routinely have tuberculosis drug susceptibility testing (DST) performed. Retreatment cases and patients not responding to antituberculosis treatment may have DST performed. Multi-drug resistant (MDR) tuberculosis, defined as *M.tb* resistant to RH, is treated with less effective agents for up to 18 months after sputum conversion. Treatment is often individualised and regimes last up to 24 months.

ART is a life-long multi-drug regimen. In patients infected with HIV-1, millions of virions are produced daily, and the reverse transcriptase target mutates rapidly. Hence, initial therapies with single or dual nucleoside reverse transcriptase inhibitors (NRTI) such as zidovudine (AZT) and didanosine (ddI) were only partially effective and rapidly led to viral drug resistance (Erice 1993). Effective therapy only became possible when non-nucleoside reverse transcriptase (NNRTI) and viral protease inhibitor (PI) drugs were developed. Combinations of

these three drug classes lead to prolonged suppression of HIV-1 replication and ultimately to a degree of immune recovery. Adherence to ART is crucial to successful viral suppression, which is very closely related to immune restoration and survival (Nachega 2007).

In South Africa (SA ART 2006), first-line ART is stavudine (d4T), lamivudine (3TC) and either nevirapine or efavirenz (NVP, EFZ). EFZ is preferred in patients receiving rifampin-based antituberculosis treatment. Patients with a CD4+ count less than 200cells/ μ L and/or a history of a WHO stage 4 illness are eligible to commence ART (SA ART 2006). In patients diagnosed with tuberculosis, ART is deferred if there is no history of a WHO Stage 4 illness and the CD4+ count is greater than 200 cells/ μ L. If there is a history of a WHO Stage 4 illness and/or the CD4+ count is less than 200 cells/ μ L, ART is commenced 2 months after initiating antituberculosis treatment. If the CD4+ count is less than 50 cells/ μ L or a serious HIV-1 related illness exists, ART may be commenced two weeks after initiating antituberculosis treatment (SA ART 2006).

Despite the availability of these antituberculosis and antiretroviral therapies, many people are still dying in South Africa. This occurs i) because the drugs are ineffective against resistant organisms, and ii) because substantial difficulties are encountered with combined concurrent treatment (Pepper 2007).

2.2 Drug resistance

Drug resistant tuberculosis

Drug resistant *M.tb* is an important reason for deterioration during antituberculosis treatment. Exogenous re-infection with MDR- *M.tb* in patients with advanced HIV-1 infection was first reported in 1993 (Small 1993). Recently, extensively drug-resistant (XDR-) tuberculosis – defined as MDR plus resistance to at least a quinolone and a second line injectable drug (CDC 2006a, CDC 2006b) – was found to be a cause of death in patients co-infected with tuberculosis and HIV-1 in a rural area of kwaZulu Natal (Gandhi 2006). Exogenous re-infection was implicated as two-thirds of patients were recently hospitalised before the onset of XDR-TB. Genotyping of the isolates showed that 85% of the patients had similar strains. The extent of transmission of drug resistant *M.tb* in Khayelitsha, Cape Town has only just been determined. A retrospective study from 2005 to 2007 uncovered a three-fold increase in the case-load of patients with MDR-TB and HIV-1 (Virginia de Azevedo, City of Tygerberg- personal communication). A similar concomitant increase among HIV-1 uninfected patients was not documented.

The continued emergence of drug-resistant *M.tb* (Shah 2007) may be due to several factors: W-Beijing strains of *M.tb* are associated with HIV-1 and have a greater propensity to become drug resistant (Rad 2003). Biologically these strains are postulated to interact in an immune subverting manner because they produce an immunosuppressive phenolic glycolipid (Reed 2004). Tuberculosis/HIV-1 co-infection is also associated with significantly reduced rifampin drug levels, which may allow the selection of drug-resistant *M.tb* bacilli (Gurumurthy 2004, Sahai 1997). Patients receiving MDR-TB treatment may also be at risk of XDR- TB. Current MDR-TB treatment is prolonged, poorly tolerated and less effective than first-line antituberculosis treatment. Worryingly, nosocomial acquisition of XDR-*M.tb* has recently been

documented (Gandhi 2006). In order to combat the expanding tuberculosis pandemic and prevent the spread of drug-resistant *M.tb*, the following are required: Rapid diagnostic methods to ascertain drug resistance (Rattan 1998), functional socio-political infrastructures, a substantial financial commitment by both first and third world countries, and new antituberculosis drugs.

Drug-resistant bacteria

Bacterial pneumonia is more common in patients with advanced HIV infection (Tumbarello, 2001). Exposure to beta lactam agents, multiple hospitalizations and low CD4+ cell counts are all risk factors for the acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA)(Tumbarello 2002). Profound immune suppression is an important risk factor for community acquired pneumonia, while prolonged hospitalisation significantly increases the risk of nosocomial bacterial pneumonia (Hirschtick 1995). *S. pneumonia* and methicillin-sensitive *S. Aureus* are frequently cultured from patients with community acquired bacterial pneumonia (Hirschtick 1995) while *Pseudomonas aeruginosa* and MRSA are cultured from those with nosocomial bacterial pneumonia (Franzetti 2006). The incidence of bacterial infections has declined among HIV infected patients in the developed world.

Chemoprophylaxis with trimethoprim–sulfamethoxazole was associated with a 67% reduction in confirmed episodes of bacterial pneumonia(Hirschtick 1995). ART reduces the incidence of bacterial infections by restoring immune function (Deeks 1997) and reducing AIDS-defining illnesses (Mouton 1997). This indirectly reduces the length of hospitalisation and results in a reduction of nosocomial bacterial pneumonia (Mouton 1997).

Drug-resistant HIV-1

The prevalence of multi-drug-resistant HIV-1 is increasing (Turner 2006). This may be explained partly by the transmission of HIV-1 from ART-experienced to HIV-1 uninfected individuals (Wensing 2005). Whilst drug resistance (DR) is not associated with increased virulence (Brenner 2002), DR is the leading cause of treatment failure amongst patients infected with HIV-1 (Lorenzi 1999). Fortunately, this is not yet a significant problem in sub-Saharan Africa. High adherence (>95%) to ART reduces viral replication, which limits the emergence of drug-resistance mutations (Burman 2005). Intermediate ART adherence (70–90%), as well as ART mono-/ dual therapy, increases the incidence of DR (Nachega 2007). Recent data suggest that moderate adherence to NNRTIs leads to sustained viral suppression (Nachega 2007). Certain reverse transcriptase mutations conferring resistance to NRTI (K65R, M184V) are associated with reduced viral fitness (Turner 2004), whilst mutations conferring NNRTI resistance are usually neutral. Interestingly, NNRTI hypersusceptibility is more common amongst viruses from NRTI experienced/ NNRTI-naïve patients compared with viruses from NRTI/ NNRTI-naïve patients, suggesting that mutations in NRTI-resistant viruses confer structural conformational advantages for NNRTIs at the NNRTI-binding site (Whitcomb 2002). Reduced viral fitness of DR HIV-1 strains allows their growth to be exceeded by wild-type HIV-1 strains (Brenner 2004). The detection of DR HIV-1 in ART-naïve patients may consequently be difficult, as standard population-based genotyping methods only detect viral populations that are greater than 20% of the total HIV-1 population (Brenner 2004); drug pressure allows the subsequent detection of DR strains (Turner 2006). In addition, DR is increased when a failing ART regimen is maintained (even though CD4+ counts are stable and viral loads are preserved over time) and may limit future treatment options (Kantor 2004, Cozzi-Lepri 2007). DR-HIV-1 is currently not a major problem in South Africa. It is thus

imperative to prevent this possibility from occurring. South Africa can ill afford the dual epidemics of MDR-HIV-1 and MDR-TB.

2.3 Difficulties associated with combining current antituberculosis and antiretroviral treatments.

Antituberculosis and antiretroviral treatment is complicated by shared drug toxicities, drug interactions, high pill burdens, and the immune reconstitution inflammatory syndrome. Co-morbid illnesses and the treatment of latent tuberculosis infection provide additional challenges.

Shared drug toxicities

Concomitant therapy for HIV-1 and tuberculosis is associated with increased risks of adverse drug effects such as nausea, gastrointestinal tract disturbance, peripheral neuropathy, cutaneous reactions, renal toxicity and potentially fatal liver toxicity (Dean 2002). Certain drug combinations should be used with caution (Table 2.1). These toxicities may necessitate therapy discontinuation, which exacerbates immune suppression and predisposes to other opportunistic infections. Shared drug toxicities also compromise adherence to the treatment regimen, leading to suboptimal treatment, and increasing the possibility of drug resistance.

Drug interactions

Numerous concurrent diseases occur in profoundly immune-suppressed HIV-1 infected patients with tuberculosis. They include *Pneumocystis jiroveci* pneumonia, toxoplasmosis, Kaposi's sarcoma, deep vein thrombosis, seizure disorders and sepsis (Ansari 2002). The potential for drug interactions increases when treatment for these illnesses is prescribed concomitantly with antituberculosis and antiretroviral treatment (Figure 2.1).

Table 2.1: Shared drug toxicities in HIV-1 / tuberculosis patients

Side-effect	Anti-retrovirals	First-line TB drugs	MDR-TB drugs	Other drugs	Other HIV disease
Peripheral neuropathy	d4T, ddI, ddC	H, E (rare)	Cy, Te, FQ, Et, Ka, Am, Lin		HIV itself
Hearing loss		S	Ka, Am, Cpr, Clr		
Vertigo		S	Ka, Am, Clr		
Optic neuritis		E, H (rare)	Et (rare), PAS (rare), Lin		
Seizures		H	Cy, Te, Of, Ci [and other FQ – rare]		
Psychosis	EFV	H	Cy, Te, Of, Ci, [other FQ] Et	TMP-SMX, steroids	HIV itself
Depression	EFV		Cy, Te, Of, Ci, Et	TMP-SMX	HIV itself
Nausea & vomiting	AZT, ddI, PI: IDV, amprena-vir (other PIs)	Z, Rfm, H, E	Et, Of, Ci, [other FQ, PAS, Clf, Lin	TMP-SMX, Amphotericin B	OI, IRIS
Gastritis		E, Z	Et		
Transaminitis	NVP, PI, EFZ	Z, R, H	Of, Ci [other FQ], Et, Cy, Te, PAS, Clr	TMP-SMX, Azoles	OI
Hepatic steatosis	d4T				OI, IRIS
Cholestasis		R	Clr	TMP-SMX	AIDS cholangiopathy
Nephrotoxicity	TDF (Fanconi syndrome)	S, R (interstitial nephritis & GN), Z+H also rarely cause interstitial nephritis	Ka, Am, Cpr, FQ – rarely cause IN, PAS causes crystalluria	Amphotericin B, TMP-SMX (interstitial nephritis)	HIVAN
Hypokalaemia	TDF		Ka, Am, Cpr	Amphotericin B	
Renal calculi	IDV			TMP-SMX	
Arthralgias and gout		E, Z, H, R	Of, Ci, [other FQ, PAS]	Thiazide (gout)	HIV itself
SJS/ TEN	NNRTIs	R, H (both rarely)	Thiacetazone, Clr, PAS, FQ	TMP-SMX	
Skin rash	NNRTIs, ABC, PI	Z, R, H, S, E	FQ, Clf, PAS, Clr, Cpr, Et, Cy, Te	TMP-SMX	Folliculitis & asteatosis
Hypersensitivity	ABC	R, S		TMP-SMX	
Leucopenia, anaemia	AZT, 3TC	R, H, Z(sideroblastic anaemia), RHZE rarely cause thrombocytopenia	FQ, strep, Cy (megaloblastic), PAS, Lin, Cpr, Clr (last 4 also thrombocytopenia)	Ganciclovir, TMP-SMX, Amphotericin B	HIV itself
Lactic acidosis	d4T, ddI, ddC, AZT		Lin		
Pancreatitis	ddI, d4T, ddC	H	Lin, Clr	TMP-SMX, steroids	
Insulin resistance	PI esp IDV				
Hyperlipidaemia	PI (except ATV), d4T			steroids	
Lipoatrophy	d4T				
Lipodystrophy	PIs				
Hepatitis B flare if drug discontinued	TDF, FTC, 3TC				
Teratogenic	EFV	S	Et		
Osteoporosis	TDF				

- Antiretrovirals: D4T = stavudine, ddI = didanosine, ddC = zalcitabine, Efv = efavirenz, AZT = zidovudine, IDV =indinavir, PI = Protease inhibitor, NVP = nevirapine, TDF = tenofovir, NNRTI = Efv + NVP, ABC = abacavir, ATV = atazanavir
- First-line TB drugs: Rfm = rifamycins, R = rifampin, H = isoniazid, E = ethambutol, Z = pyrazinamide, S = streptomycin
- MDR-TB drugs: Cy = cycloserine, Te = Terizidone, Of = Ofloxacin, Ci = Ciprofloxacin, Et = Ethionamide[+prothionamide], Ka = Kanamycin, Am = Amikacin, FQ = fluoroquinolones, Lin = linezolid, Cpr = capreomycin, Clr = clarithromycin, Clf = clofazimine, PAS = p-amino-salicylate
- TMP-SMX = trimethoprim sulfamethoxazole, IRIS = immune reconstitution inflammatory syndrome, OI = opportunistic infections
- Drugs **highlighted in blue** should not be used simultaneously because they potentiate the drug side-effect or are associated with significantly increased morbidity or mortality.
- References: Burman 1999, Burman 2005, CDC 2007, Calza 2004, Dean 2002, Feve 2004, Kaplan 2008, von Hentig 2008

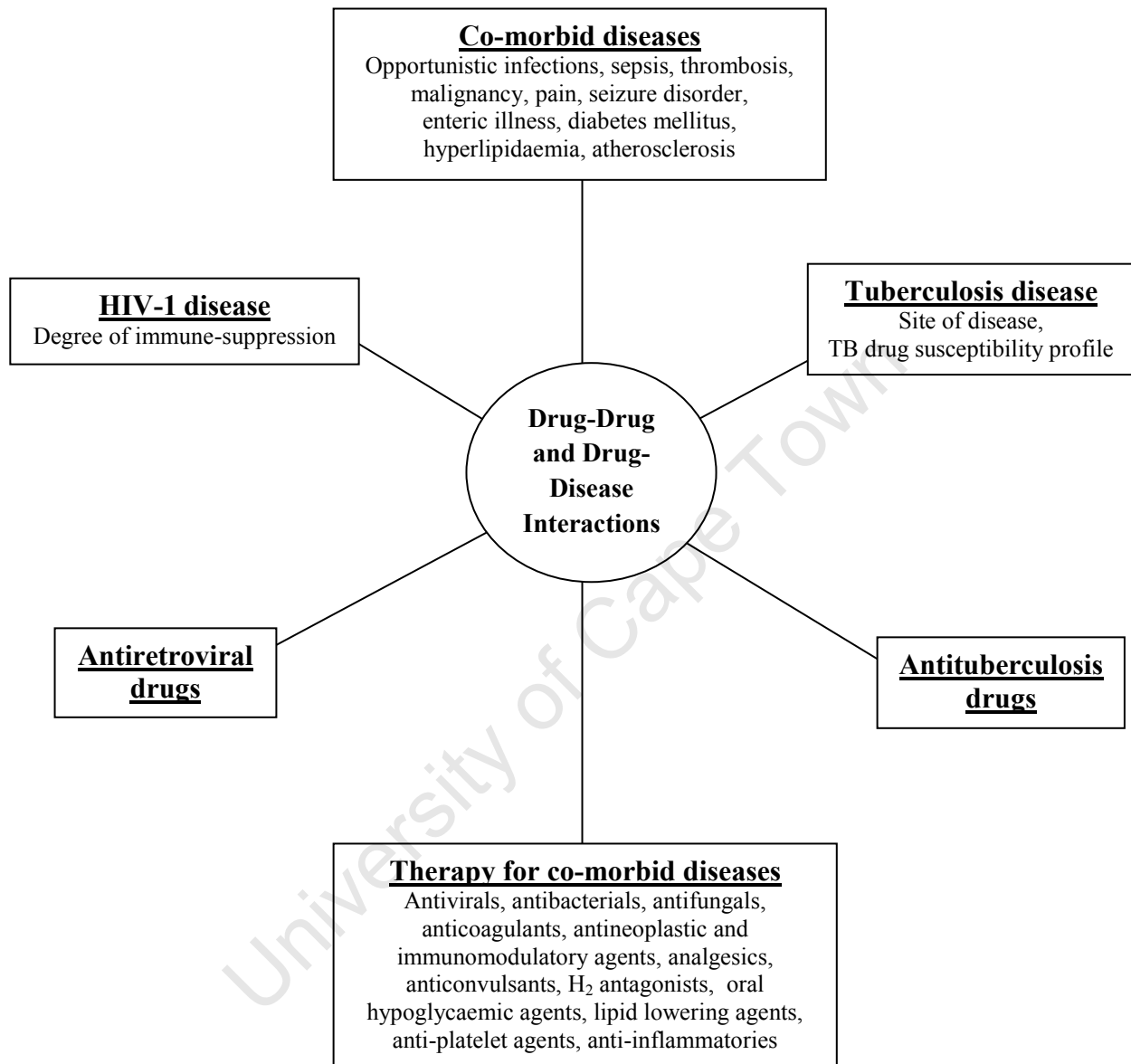


Figure 2.1: Potential contributors to drug-drug and drug-disease interactions: tuberculosis, HIV-1 and co-morbid diseases and therapy

While ART partially restores immune function and prolongs life, HIV-1 infected patients are at increased risk of metabolic and vascular disorders; these disorders may occur as a direct result of HIV-1 infection (Kaplan 2008), as side-effects of ART (von Hentig 2008, Calza 2004, Feve 2004), or because prolonged survival allows HIV-1 infected patients to develop diseases that would otherwise occur later in life. As the two epidemics of HIV-1 and metabolic syndrome unfold in Africa, simultaneous treatment for HIV-1, diabetes mellitus and diseases due to atherosclerosis will challenge many clinicians. Furthermore, a subgroup of patients will still have an increased risk of tuberculosis recurrence (Lawn 2006) and require additional treatment with rifamycins.

Potential drug-interactions should always be considered in HIV-1 infected patients with tuberculosis, especially as many drugs utilized to treat HIV-1, tuberculosis and co-morbid diseases are either inducers, inhibitors or substrates of cytochrome p450 (CYP) – (tables 2.2, 2.3, 2.4). Drugs that induce a particular CYP iso-enzyme increase the rate at which the CYP iso-enzyme metabolizes its substrate to metabolites. Conversely, inhibitors decrease the rate of metabolite production resulting in increased substrate. Rifampin is a potent inducer of CYP3A4 whereas ritonavir is an inhibitor of CYP3A4. Suboptimal anticoagulation and contraceptive failure may occur when warfarin and oral contraceptives, respectively, are co-administered with CYP inducers. Similarly, rhabdomyolysis, symptomatic hypotension and excessive sedation may result when statins, calcium antagonists and benzodiazepines, respectively, are co-administered with CYP inhibitors.

Table 2.2: Substrates, inhibitors and inducers of cytochrome p450 enzymes (CYP), and P-glycoprotein (PGP)			
	Substrates	Inhibitors	Inducers
CYP1A2	Acetaminophen Caffeine Clozapine Phenacetin R-warfarin Theophylline	Erythromycin Cimetidine Ciprofloxacin Ritonavir	Cigarette smoke Phenobarbital Phenytoin Rifampin
CYP2C9/10	Dapsone Phenytoin S-Warfarin Tolbutamide	Erythromycin Amiodarone SMX/TMP Isoniazid Metronidazole Fluconazole	Carbamazepine Rifampin Phenobarbital Phenytoin
CYP2C19	Benzodiazepine Proton pump inhibitors	Cimetidine Diazepam Fluoxetine Omeprazole Isoniazid	Rifampin Phenobarbital Phenytoin
CYP2D6	Amiodarone Amitriptyline Clomipramine Clozapine Codeine Desipramine Fluoxetine Haloperidol Imipramine Metopropolol Propanolol Risperidone Timolol	Amiodarone Cimetidine Fluoxetine Fluphenazine Haloperidol Quinidine Ritonavir	
CYP2E1	Halothane Acetaminophen		Ethanol Isoniazid Phenobarbital
CYP3A4	Carbamazepine Cyclosporin Dapsone Diltiazem Erythromycin Lidocaine Midazolam Nifedipine Quinidine Simvastatin Tacrolimus Tamoxifen Testosterone Valproic acid Verapamil	Amiodarone Cimetidine Clarithromycin Erythromycin Fluconazole Fluoxetine Grapefruit juice Indinavir Itraconazole Ketonazole Ritonavir Sertraline	Carbamazepine Dexamethasone Phenobarbital Phenytoin Rifampin
PGP	Quinolones Itraconazole Digoxin	Cyclosporine Tacrolimus	Rifampin
Abbreviations: SMX-TMP, trimethoprim-sulfamethoxazole References: Burman 1999, CDC 2007, Deeks 1997, Rae 2001, Ribera 2001			

Table 2.3: Pharmacokinetics of antituberculosis drugs

Drug Name	CYP			P-glycoprotein			Drug metabolism (other than CYP)	Percentage (%) protein binding, principal protein bound to
	Substrate	Inducer	Inhibitor	Substrate	Inducer	Inhibitor		
1st line TB drugs								
Rifampin	No	3A4, 1A2, 2C, 2D6			Yes		Metabolized by deacetylation induces UDPGT and sulphotransferase	85% protein bound
Rifapentine		3A4, 2C8/9						97% protein bound
Rifabutin	3A	3A, 2D						Least protein bound -70%
Isoniazid	No	2E1	2C9, 2C19				Metabolized by NAT2 and 2E1 Inhibits MAO	
Pyrazinamide	No	No					Metabolite inhibits uric acid secretion by renal tubules	
Ethambutol	No	No					15% metabolised to aldehyde and dicarboxylic metabolites	
Streptomycin	No	No	No				No identified metabolites	
Second line drugs								
Kanamycin	No	No	No				No identified metabolites	
Amikacin	No	No	No				No identified metabolites	
Ofloxacin				Yes				
Ciprofloxacin			1A2	Yes				20 – 40%
Moxifloxacin				Yes				40 – 50%
Ethionamide	3A							
Capreomycin								
Clarithromycin	3A		3A1				Hydroxylation and oxidative N- demethylation >50% acetylated	
PAS								
Augmentin								
Linezolid							Metabolised by non-enzymatic oxidation, reversible inhibitor of MAO A/ B	31%, albumin
Cycloserine								
Terizidone								
Abbreviations: TB = tuberculosis, CYP = cytochrome p450 iso-enzyme, PAS = para-aminosalicylic acid, UDPGT = uridine diphosphate glucuronyltransferase, MAO = monoamine oxidase, NAT2 = N-acetyltransferase 2 References: Burman 2001, Iatsimirskaia 1997, Jamis-D 1997, Jasmer 2002, Keung 1999, Langdon 2005, Lortholary 1995, McIlleron 2006, Namdar 2005, Reith 1998, Yew 2002								

Table 2.4: Pharmacokinetics of antiretroviral drugs								
Drug Name	Substrate	CYP Inducer	Inhibitor	Substrate	P-glycoprotein Inducer	Inhibitor	Drug metabolism (other than CYP)	Percentage (%) protein binding, principal protein bound to
<u>Nucleoside Reverse Transcriptase Inhibitors</u>								
Zidovudine							Glucuronidation	34-38%
Stavudine								5%
Lamivudine							5 to 10% metabolised to inactive trans-sulphoxide metabolite	< 36%
Didanosine								5%
Zalcitabine								5%
Abacavir							alcohol dehydrogenase, glucuronosyltransferase	50%
<u>Nucleotide Reverse Transcriptase Inhibitor</u>								
Tenofovir								
<u>Non-Nucleoside Reverse Transcriptase Inhibitors</u>								
Efavirenz	3A4, 2B6	3A4	2C9/19, 3A4	Yes				>99%, albumin
Nevirapine	3A4, 2B6	3A4, 2B6		Yes				60%, albumin
Delavirdine	3A4, 2D6, 2C9/19		3A4, 2C9, 2C19, 2D6	Yes				98%, albumin
<u>Protease Inhibitors</u>								
Amprenavir	3A4, 2D6		3A4	Yes				90% α -acid glycoprotein
Atazanavir	3A4		3A, GT	Yes				89%, α -acid glycoprotein 86%, albumin
Darunavir	3A4		3A4	Yes			Hydroxylation, hydrolysis	95% α -acid glycoprotein
Indinavir	3A4, GT		3A4	Yes				60%, α -acid glycoprotein
Lopinavir	3A4	GT	3A4, 2D6	Yes				98-99% α -acid glycoprotein
Nelfinavir	3A4, 2C9 , 2C19, 2D6	GT	3A4	Yes				>98%, α -acid glycoprotein
Ritonavir	3A4, 2D6	GT, 1A2, 3A, 2C9	3A4, 2D6	Yes		Yes		98-99%, α -acid glycoprotein
Saquinavir	3A4		3A4	Yes				98%, α -acid glycoprotein
Tipranavir	3A4	3A4		Yes	Yes		glucuronidation	>99%, α -acid glycoprotein
<u>Fusion Inhibitor</u>								
Enfuvirtide							NADPH hydrolysis	92%, albumin
Abbreviations: CYP = cytochrome p450 iso-enzyme, GT = glucuronyl transferase, NADPH = Nicotinamide adenine dinucleotide phosphate References: CDC 2007, Deeks 1997, Gallicano 1999, King 2004, King 2006, LeTiec 2005, McIlleron 2006, Patel 2005, Rittweger 2007, Smith 2001, Veal 1995, Vella 1998								

Rifamycins form the backbone of regimens for drug-susceptible *M.tb* because they reduce the duration of therapy, are highly effective, and are less likely than other antituberculosis drugs to select out resistant strains in multi-drug regimens (Rattan 1998). However, through its activation of a master transcriptional regulator, the pregnane X receptor (PXR), rifampin induces the expression of a broad array of enzymes and drug-transporting molecules including cytochrome P450 (CYP) 3A and CYP2C iso-enzymes, CYP2D6, CYP2B6 and p-glycoprotein, amongst others (Rae 2001). Thus, repeated doses of rifampin result in clinically important reductions in the levels of many drugs, such as PIs, NNRTIs, trimethoprim and sulfamethoxazole (the latter two co-formulated in co-trimoxazole widely used for prophylaxis in patients with advanced HIV-1 infection) (CDC 2007, Ribera 2001). The co-localization of p-glycoprotein and CYP enzymes in enterocytes, hepatocytes and renal tubular cells may enhance the effects of rifampin on common substrates, such as PIs, causing more extensive pre-systemic metabolism and accelerated drug elimination. The clinical consequences of rifampin-related decreases in serum concentrations of the anti-retroviral drugs have not been fully studied, but they potentially lead to loss of anti-viral efficacy and stepwise accumulation of resistance mutations (de Requena 2005, Marzolini 2001, Dumon 2000, Masquelier 2002). Whilst NNRTIs, besides delavirdine, can be given with rifampin (Borin 1997), dose increases may be necessary. With the exception of ritonavir-boosted PIs, all other PIs are contraindicated with rifampin (Burman 1999). Certain ritonavir-boosted PIs can be used with rifampin, but increased doses of ritonavir or a higher dose of the companion PI are required. Rifapentine, a rifamycin with a longer half-life, allows intermittent administration that may simplify therapy. Rifapentine is also a less potent inducer of CYP than rifampin. However, rifapentine has not been widely introduced as it is associated with increased rates of TB drug resistance when used once weekly during the continuation phase of antituberculosis treatment (Vernon 1999). Intermittent rifampin therapy in the continuation phase is also associated with rifampin

resistance in patients with advanced HIV-1 infection (Burman 2009). Rifabutin, the rifamycin which induces CYP enzymes the least, is safe to use with most NNRTIs and PIs, except delavirdine and saquinavir. However, it is also a substrate for CYP3A4 (Burman 2001). Thus, its serum concentration and toxicity are increased when co-administered with PIs and decreased when co-administered with efavirenz. Rifabutin dose adjustments are required in these settings (CDC 2007). Rifabutin and the anti-retroviral nevirapine can be given together at standard doses, but delavirdine is contraindicated with rifabutin (CDC 2007). Also, the cost of rifabutin precludes its use in the developing world. Although they are of lesser importance, other pharmacokinetic interactions may confound combined treatments. Isoniazid is an inhibitor of CYP2C19 and CYP3A, PIs inhibit CYP3A, CYP2D6 and p-glycoprotein, and efavirenz and nevirapine induce the expression of CYP3A4 and CYP2B6. Other commonly co-administered drugs also cause important changes in anti-retroviral concentrations through induction or inhibition of CYP iso-forms (e.g. anticonvulsants like carbamazepine and phenytoin may decrease, and azole anti-fungals, macrolide antibiotics and H2 antagonists may substantially increase PI or NNRTI concentrations).

Pharmacodynamic interactions further complicate combined treatment of tuberculosis and HIV-1. Unanticipated hepatotoxicity has been reported in healthy volunteers receiving dose-adjusted PIs in combination with rifampin (La Porte 2004, Grange 2005). Interestingly, repeated doses of rifamycins before the introduction of the PIs appear to be associated with a higher risk of hepatotoxicity than the introduction of rifampin after establishing regular doses of the PIs. Although not adequately studied, such high rates of hepatotoxicity have not been reported in patients receiving increased doses of PIs with rifampin-based antituberculosis regimens, indicating that disease-related modulation of hepatotoxicity can occur. The effect of HIV-1 infection on the concentrations of orally administered first-line antituberculosis drugs is

also a concern. It appears that HIV-1 infected patients achieve not only somewhat lower concentrations of rifampin and ethambutol, but also lower concentrations of isoniazid and pyrazinamide (Gurumurthy 2004, McIlleron 2006, Sahai 1997, Zhu 2004). Patients with more advanced HIV-1 disease and those with diarrhoea appear to be at most risk. Although these drug-disease interactions do not appear to have a marked effect on treatment outcomes, their importance has not been fully evaluated.

High pill burden

Combined therapies for tuberculosis and HIV-1, together with the recommended co-trimoxazole prophylaxis, lead to a very high pill burden – an average of 15 pills per day for 6–22 months in South Africa (fixed dose combinations (FDC) for antituberculosis medication but not ART). Furthermore, in high burden countries standard treatment approaches are adopted and FDCs are often used to reduce the pill burden and to simplify drug supply, prescribing and administration. Pharmacokinetic interactions necessitating dose adjustment of individual drug components complicate treatment delivery, especially in the context of standardised approaches using FDCs. Thus, optimisation of therapy inevitably leads to a greater pill burden.

Immune reconstitution inflammatory syndrome

Immune reconstitution inflammatory syndrome (IRIS), which results from dysregulated immune recovery (French 2000), occurs in severely immune-suppressed HIV-1 patients typically one to four weeks after ART initiation (Breton 2004, Narita 1998). In tuberculosis-related IRIS, an exuberant inflammatory reaction is directed towards mycobacterial antigens (Bourgarit 2006). This causes worsening pulmonary infiltrates, pleural effusions, lymphadenitis and potentially fatal neurologic tuberculomata (Breton 2004, Narita 1998). Risk factors for TB-IRIS include a low baseline CD4+ count, a high baseline viral load, a short interval from

antituberculosis treatment to ART initiation, and disseminated tuberculosis (Breton 2004, Shelburne 2005). Determining the optimal time to initiate ART in severely immune-suppressed tuberculosis patients is difficult (Dean 2002, Burman 2001). Early initiation of ART may increase the risks of TB–IRIS, non-adherence, drug toxicities and drug interactions, but the risks of death, other opportunistic infections (OIs) and malignancies may be greater if ART initiation is delayed (Lawn 2007). Corticosteroid treatment for TB–IRIS is sometimes recommended, but such immunosuppressive therapy may be associated with reactivation of occult OIs and malignancies, such as CMV and Kaposi’s sarcoma. Recently, a randomised trial of corticosteroids vs. placebo for TB–IRIS demonstrated the benefit of corticosteroids in reducing hospital admissions and improving symptoms (Meintjes 2009, Conference of Retroviruses and Opportunistic Infections [CROI]). However, a substantial proportion of patients with TB-IRIS develop life-threatening infections. Furthermore, up to 10% of patients presenting with TB-IRIS may have drug resistant *M.tb* (Meintjes 2009, CID). The benefit of corticosteroids always needs to be weighed against the risks of exacerbating occult infections.

Co-morbid illnesses

HIV-1 induced immunosuppression, predisposing to OIs and malignancies, coupled with antituberculosis/ antiretroviral drug side effects and TB–IRIS can give rise to multiple pathologies in certain organs, especially the liver (Table 2.2). When faced with significant biochemical or clinical evidence of hepatitis, it is a dilemma whether to withdraw all drugs or the most likely offending candidates. Evidence certainly suggests withdrawal of rifampin, isoniazid, pyrazinamide and NNRTI medications, the latter under the cover of a two NRTI ‘tail’ to reduce the risk of NNRTI resistance. Substituting ‘liver friendlier’ alternatives such as streptomycin and quinolones to continue the treatment of tuberculosis is of uncertain efficacy. Determining the cause of the hepatitis without the use of invasive techniques, such as

endoscopic retrograde cholangio-pancreatography or biopsy, poses major challenges to clinicians in resource-limited settings. A further problem on resolution of the hepatitis is when and how drugs should be re-introduced. Consensus is that no attempt be made to re-introduce nevirapine therapy after significant drug-induced toxicity, but it is common experience that all anti-tuberculosis drugs may be re-introduced. This is usually done sequentially with monitoring of clinical signs and hepatic enzymes. But this is a prolonged empirical exercise that maintains the patient in expensive hospital care and at risk of nosocomial infection. Well-conducted clinicopathologic studies with adequate numbers of patients are required to address these issues and inform practice.

Table 2.2: Causes of liver disease in patients with tuberculosis and HIV-1

	Drugs	Infections	Malignancies
<i>Transaminitis (ALT > 3 times normal)</i>			
	TB drugs: Rif, INH, Z ART: NNRTI Azoles TMP-SMX	Viral Hepatitis A,B,C CMV EBV	
<i>Canalicular Pattern (ALP > 3 times normal)</i>			
- Infiltration	NRTI (steatosis)	Granulomatous hepatitis <i>Mycobacterium tuberculosis</i> / <i>Mycobacterium avium-intracellulare</i> / TB-IRIS Fungal CMV	Kaposi's sarcoma Lymphoma
- Cholangiopathy (USS liver/ ERCP)		CMV <i>Salmonella</i> , <i>Campylobacter</i> , <i>Isospora belli</i> , <i>Microsporidium</i> , <i>Cryptosporidium</i>	
- Enzyme induction	Macrolides		
Protease inhibitors can cause unconjugated hyperbilirubinaemia Sepsis causes transaminitis with conjugated hyperbilirubinaemia If non-specific hepatitis (ALT < 3x normal), then consider malnutrition, NRTIs, ethanol, herbal medication and viral hepatitis serology			

Treatment of HIV-1-associated latent tuberculosis infection

ART reduces the incidence of tuberculosis, though it remains to be seen whether this benefit may be offset by increased survival and an overall increase in lifetime risk. Treatment of latent tuberculosis infection (LTBI) also reduces the risk of subsequent tuberculosis in HIV-1 infected people, though the duration of protection is relatively short (Woldehanna 2004). It therefore appears logical to potentially combine therapies to prevent tuberculosis. However, the risk-benefit profile of preventive antituberculosis treatment in these circumstances is unknown: There are significant overlapping hepatic and neurological toxicities (as outlined above). In addition, the most effective preventive therapy is isoniazid monotherapy, but this may be associated with an increased risk of isoniazid resistance if inadvertently given to patients with active disease (de Jong 2004). Therefore, whilst the prescribing of either isoniazid or ART has significant benefits in HIV-1 infected persons, a randomised controlled trial is still needed to determine the efficacy and risks of combination isoniazid and ART. The combination regimen of rifampin and pyrazinamide for two months was associated with liver toxicity, even among HIV-1 uninfected persons, and is no longer recommended (American Thoracic Society 2000). There is very little evidence to guide prescription of preventive therapies in persons exposed to MDR-*M.tb* (Jasper 2002).

2.4 Conclusion

Substantial challenges exist during antituberculosis treatment in profoundly immune-suppressed patients. These challenges relate to multi-drug therapy, high pill burdens, drug resistant pathogens, complex drug interactions and IRIS. Despite the incessant collision of tuberculosis and HIV, the entity of clinical deterioration during antituberculosis treatment is poorly characterised in sub-Saharan Africa. In the following chapters, I relate my previous 3 years of experience with this intriguing entity.

Chapter 3

Clinical Deterioration during Antituberculosis Treatment at a District Hospital in South Africa: The importance of drug resistance and AIDS-defining illnesses

University of Cape Town

Contributions: The study was conceived and designed by the candidate, Chelsea Morroni, Robert J. Wilkinson and Graeme Meintjes. Data was obtained by the candidate, Kevin Rebe, Robert J. Wilkinson and Graeme Meintjes. The data was analysed by the candidate and Chelsea Morroni. The candidate compiled the initial draft which was critically revised by Kevin Rebe, Chelsea Morroni, Robert J. Wilkinson and Graeme Meintjes.

Publications: **Pepper DJ**, Rebe K, Morroni C, Wilkinson RJ, Meintjes G. Clinical deterioration during antituberculosis treatment at a district hospital in South Africa: the importance of drug resistance and AIDS defining illnesses. *PloSONE* **2009**; 4(2):e4520

This study determined the causes and frequency of clinical deterioration during antituberculosis treatment at an urban referral hospital in a high HIV-1 prevalence setting.

3.1 Methods

Setting

A prospective observational study conducted from 9 January to 8 April 2007 at GF Jooste Hospital. This is an urban 200-bed adult (≥ 15 years age) referral hospital in Cape Town, South Africa that serves 1.3 million people and receives 8,000 referrals per month (Burch 2006) from 30 primary care clinics. The national HIV-1 and Tuberculosis Programmes were described in Chapter 2. By April 2007, over 10,000 people had initiated ART within the hospital's catchment area (Meg Osler, Provincial Government of the Western Cape- personal communication).

Study procedures

Eligibility. We assessed adult patients (≥ 15 years) who received ≥ 14 days of antituberculosis treatment and were referred by a medical doctor or nurse to our hospital's Emergency

Department or Infectious Diseases Unit. Prior to referral, patients were diagnosed with tuberculosis at any of the 12 tuberculosis clinics within the hospital's catchment area. Patients commenced antituberculosis treatment at their tuberculosis clinic and if they deteriorated or did not stabilise on treatment they were referred to our hospital for assessment. Patients were considered eligible if clinical worsening or failure to stabilise on therapy was confirmed on clinical assessment by a medical doctor at the hospital. During the study, doctors and nurses at the clinics were not notified that the study was occurring. Patients were referred and managed according to current provincial practice, as recorded in the following paragraphs.

Tuberculosis diagnosis and referral. Microbiological confirmation of tuberculosis disease was defined as a specimen, obtained from a patient with symptoms and signs of tuberculosis, which cultured *M.tb* and/or was smear positive for acid-fast bacilli (SATB guidelines 2004). An empiric diagnosis of tuberculosis was defined as: Antituberculosis treatment initiated when the tuberculosis specimen was smear negative for acid fast bacilli and culture negative (or pending) for *M.tb* but the South African National Tuberculosis Control Programme's case definitions for smear-negative and extra-pulmonary tuberculosis were fulfilled (SATB guidelines 2004).

Data collection. Clinical data regarding tuberculosis diagnosis, antituberculosis treatment, HIV-1 status and ART were recorded. Reasons for clinical deterioration were determined by clinical assessment and laboratory investigations performed by medical doctors working at the hospital. All eligible patients were assessed at admission to hospital by attending physicians in Internal Medicine and reviewed weekly during inpatient stay by attending physicians in Infectious Diseases. We performed investigations according to clinical presentation. Investigations included C-reactive protein, full blood count (also called complete blood cell count), urea and electrolytes, serological and blood culture investigations. Patients underwent

renewed/ repeated diagnostic procedures to either confirm tuberculosis disease (if previously microbiologically unconfirmed) or to exclude drug-resistant *M.tb*. Specimens for tuberculosis microscopy, culture and sensitivity included sputum, pleural fluid, lymph node aspirates, ascitic fluid or cerebrospinal fluid (CSF). Chest radiography and computerised tomographic scanning were performed as indicated. Investigations also included sputum direct immuno-fluorescent antigen tests (DFAT) for *Pneumocystis jiroveci* pneumonia, CSF bacterial and fungal cultures, stool microscopy for coccidian parasites, and culture for bacteria. All patients who developed drug-induced hepatitis were initially managed as inpatients according to a standardised protocol, as follows. The tuberculosis diagnosis was evaluated by reviewing results of all tuberculosis specimens. After clinical stabilisation and return to baseline of liver function tests, patients with non-life threatening hepatitis and microbiologically proven tuberculosis were monitored and antituberculosis drugs were sequentially re-introduced. In severe cases (coagulopathy or encephalopathy), rechallenge with antituberculosis treatment causing the hepatitis was not attempted. Instead, patients were treated with an alternative antituberculosis regimen.

Tuberculosis diagnostics. The South African National Health Laboratory Services performed tuberculosis diagnostics. Ziehl-Nielsen staining was performed within 24 hours on all tuberculosis specimens, while auramine staining was performed on broth culture isolates. *M.tb* liquid culture, solid media susceptibility testing for isoniazid and rifampin, and quality assurance were performed, as described by Barnard *et al* 2008. According to provincial protocol ethambutol (7.5 mcg/mL), ethionamide (20 mcg/mL), amikacin (30 mcg/mL), kanamycin (6 mcg/mL) and ofloxacin (2 mcg/mL) susceptibility testings were only performed in patients with rifampin resistance. Susceptibility testings to pyrazinamide, streptomycin and

the remaining three second line drugs—terizidone, capreomycin, para-aminosalicylic acid—were not performed.

Definitions.

Positive cultures for *M.tb* were categorised on the basis of drug susceptibility as: (i) susceptible to both isoniazid and rifampin; (ii) mono-resistant to rifampin; (iii) resistant to at least isoniazid and rifampin (MDR- *M.tb*); (iv) resistant to isoniazid, rifampin and either resistant to an injectable agent (amikacin or kanamycin) or ofloxacin (pre-XDR *M.tb*); and (v) resistant to isoniazid, rifampin, ofloxacin, and an injectable agent (XDR *M.tb*). ‘Rifampin resistance’ was defined as any resistance to rifampin and included rifampin monoresistant-, MDR- , pre-XDR- and XDR- *M.tb* (WHO 2007).

Extended-spectrum beta lactamase (ESBL) producing bacteria were defined as bacteria having clavulanate-inhibited transferable enzymes able to hydrolyse third and fourth generation cephalosporins while methicillin-resistant *Staphylococcus aureus* (MRSA) had an oxacillin minimum inhibitory concentration (MIC) >4 mg/l.

TB-IRIS was defined according to a clinical case definition (Meintjes 2008). Adherence to antituberculosis treatment was assessed by patient report, tuberculosis clinic cards (upon which daily doses taken are recorded) and/or collateral information from the health care worker at the tuberculosis clinic. No specific criteria were utilised but if no subsequent cause for deterioration was found and the patient had <80% adherence, the patient was assessed as having poor adherence. An additional illness to tuberculosis was defined as a second illness in patients where the initial tuberculosis diagnosis was microbiologically confirmed or initial clinical response in smear-negative and/ or extra-pulmonary tuberculosis was observed. An

additional illness to tuberculosis excluded poor adherence to antituberculosis treatment, rifampin resistant *M.tb*, TB-IRIS or a paradoxical tuberculosis reaction. The use of concomitant ART distinguished IRIS from a paradoxical tuberculous reaction; IRIS occurred in patients receiving ART while paradoxical TB reactions occurred in the absence of ART. An alternate illness to tuberculosis was diagnosed when a clinical illness initially diagnosed as tuberculosis was not confirmed by smear or culture, there was no clinical response to antituberculosis treatment and we found evidence of an alternate illness to explain the clinical presentation and course.

Outcomes. The primary outcome of the study was reasons for clinical deterioration during antituberculosis treatment. The secondary outcome was the proportion of patients who started antituberculosis treatment and required referral to hospital for clinical deterioration

Analysis. Data analysis was conducted using STATA-10 (Stata Corporation, College Station, Texas). Descriptive statistics were employed for basic characterization of variables. Wilcoxon rank-sum tests to compare medians, and Fisher's exact test of probability to compare proportions were used, as appropriate, to identify associations. The Research Ethics Committee of the University of Cape Town approved the study (REF: 239/2007).

3.2 Results

Characterization of cohort

Three hundred and fifty-two patients met inclusion criteria during the 3-month period. At initial tuberculosis diagnosis, 163 (46%) had pulmonary tuberculosis, 67 (19%) had extra-pulmonary tuberculosis and 122 (35%) had pulmonary and extra-pulmonary tuberculosis. The median duration from initiation of antituberculosis treatment to clinical presentation was 93 days (interquartile range, IQR 43–149). Eighty-four percent of tuberculosis patients (n=296) required hospital admission for a median duration of 9.5 days (IQR 4–18). Patients admitted for deterioration during antituberculosis treatment accounted for 17% of medical admissions (296/1,755) during the three-month study period.

Eighty-three percent of tuberculosis patients (n=291) were HIV-1 infected and 9% (n= 33) were HIV-1 uninfected (Figure 3.1). HIV-1 testing was not performed in 28 patients prior to referral or during investigation at our hospital; reasons included: not offered voluntary counselling and testing (VCT), VCT declined or patient too sick to obtain informed consent. Baseline features of tuberculosis patients stratified by HIV-1 status are shown in Table 3.1. HIV-1 infected tuberculosis patients were more likely to be female, and less likely to have a past history of tuberculosis or microbiological confirmation of tuberculosis ($p=0.01$). The median CD4⁺ count among HIV-1 infected tuberculosis patients was 89 cells/ μ L (n= 270). Of 255 patients who met the South African Department of Health criteria to receive ART (SATB guidelines 2004), 122 (48%) were receiving this treatment.

Final diagnoses

An additional illness to tuberculosis was found in 72% of tuberculosis patients whose HIV-1 status was known (232/ 324, Figure 3.1). We diagnosed rifampin resistant *M.tb* (n= 41), TB-

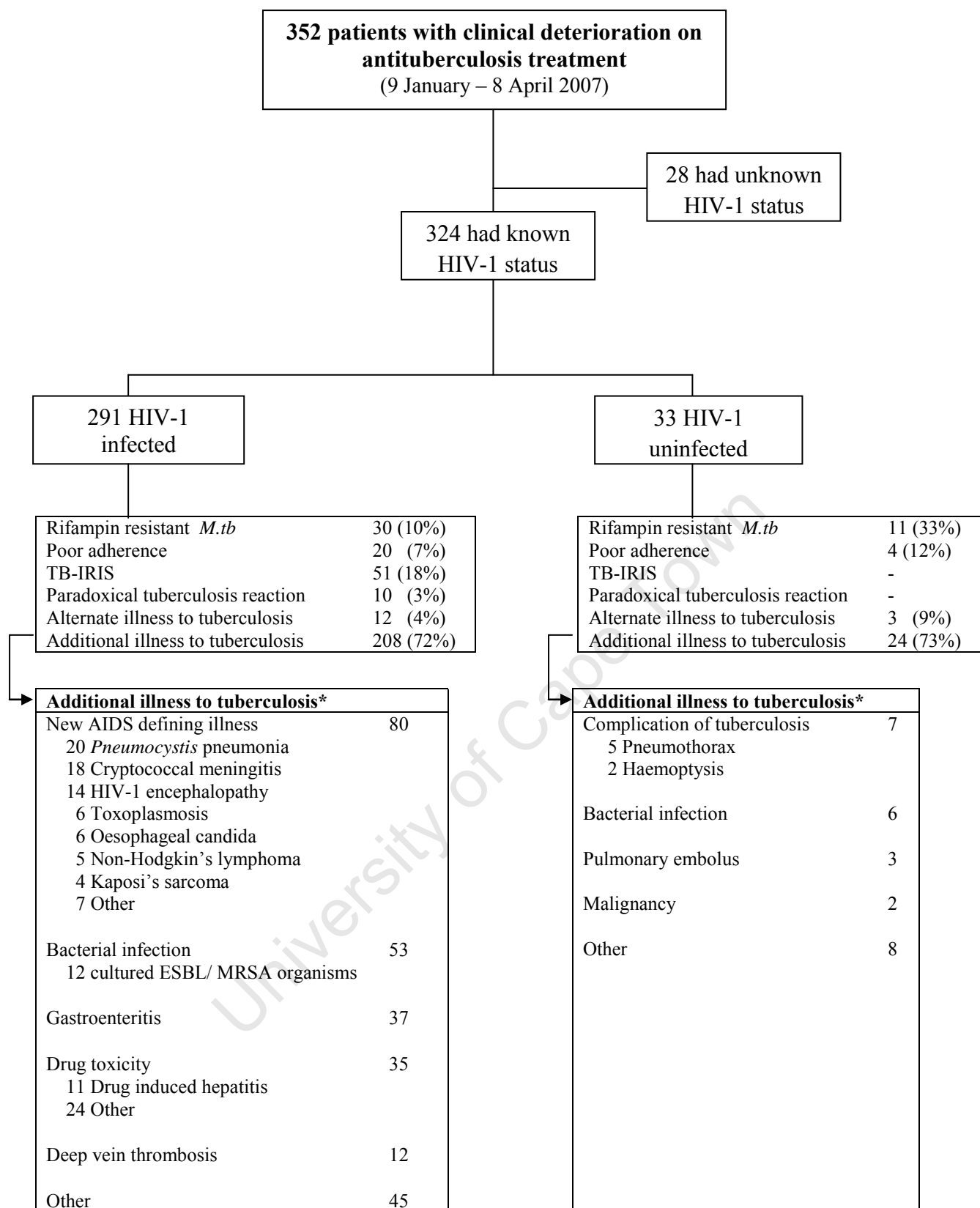


Figure 3.1: Final diagnoses in 352 patients with clinical deterioration on antituberculosis treatment: * Many patients had > 1 cause for clinical deterioration, particularly additional illnesses, HIV-1 = human immunodeficiency virus, *M.tb* = *Mycobacterium tuberculosis*, TB-IRIS = tuberculosis associated-immune reconstitution inflammatory syndrome, Paradoxical tuberculosis reaction = initial clinical improvement with subsequent recurrence of tuberculosis clinical features but no evidence of drug resistant tuberculosis/ or any other illness and patient not receiving antiretroviral therapy, MRSA = methicillin resistant *Staphylococcus Aureus*, ESBL = extended spectrum beta lactamase producing organism

Table 3.1: Characteristics of patients deteriorating on antituberculosis treatment (N=324) according to HIV-1 status

	HIV-1 infected (n=291)	HIV-1 uninfected (n=33)	P-value
Demographics			
Male – n (%)	138 (47)	24 (73)	0.006
Median age – years (range)	34 (16-86)	37 (19-68)	NS ¹
Basis of tuberculosis diagnosis			
Smear +ve – n (%)	106 (36)	22 (67)	0.001
Smear -ve / Culture +ve – n (%)	28 (10)	1 (3)	NS
Empiric diagnosis – n (%) ²	157 (54)	10 (30)	0.01
Previous tuberculosis			
– n (%)	101 (35)	19 (58)	0.01
Admitted			
– n (%)	238 (82)	31 (94)	NS
Median duration – days (IQR ³)	10 (4 – 18)	11 (5 – 22)	NS
Outcome			
Died as inpatient – n (%)	43 (15)	5 (15)	NS
Illness contributory to death			
Bacterial illness	12	1	
Enteric illness	8	-	
<i>Pneumocystis jiroveci</i> pneumonia	6	-	
Venous thromboembolism	5	2	
Drug side effects	3	-	
Rifampin resistant-tuberculosis ⁴	3	-	
Kaposi sarcoma	2	-	
Cryptococcal meningitis	2	-	
Neurologic TB-IRIS	2	-	
Chronic renal failure	-	1	
Acute coronary syndrome	-	1	
¹ NS: not significant, p-value significant at p≤0.05			
² i.e. No microbiological proof at commencement of tuberculosis treatment			
³ Interquartile range			
⁴ Rifampin resistant-tuberculosis = <i>Mycobacterium tuberculosis</i> , with resistance to at least rifampin, cultured from a patient with clinical deterioration of symptoms attributable to progressive tuberculosis disease			

IRIS (n= 51) and other drug resistant bacterial infections (n =12). Twenty-four tuberculosis patients had poor adherence to antituberculosis treatment and fifteen patients had an alternate illnesses to tuberculosis.

Additional illness to tuberculosis. Additional illnesses to tuberculosis differed according to HIV-1 status (Figure 3.1). In the HIV-1 infected group, new AIDS-defining illnesses (n= 80), bacterial infections (n= 53), gastroenteritis (n= 37) and drug toxicities due to ART/ antituberculosis/ antibiotic/ antineoplastic medications (n= 35) were most frequent. In the HIV-1 uninfected group, complications of tuberculosis, or its therapy (n= 7), and bacterial infections (n =6) were most common.

Antituberculosis drug resistance. Rifampin-resistant *M.tb* was found in 41 tuberculosis patients (Figure 3.2). Eight had rifampin mono-resistant *M.tb*, 24 had MDR- *M.tb*, four had pre-XDR- *M.tb* and five had XDR- *M.tb*. Extended sensitivity testing was performed in only 15 (37%) of the 41 rifampin-resistant tuberculosis cases; one third (5/ 15) of these patients had XDR- *M.tb*, while a further 27% (4/15) had pre-XDR- *M.tb*. Figure 2 shows *M.tb* culture results at initial TB diagnosis (left figure) and at subsequent deterioration (right figure). At initial tuberculosis diagnosis specimens were sent for tuberculosis culture in 237/352 (67%) patients, with 131 TB patients having a positive culture (Figure 3.2). Of 131 patients that cultured *M.tb* from specimens at initial tuberculosis diagnosis, 46 (35%) did not have drug susceptibility testing performed. Of the 85 TB patients who did have tuberculosis drug sensitivity analysis at diagnosis, 12 (14%) cultured rifampin-resistant *M.tb* and 73 patients (86%) cultured rifampin-sensitive *M.tb*. At clinical deterioration, specimens were sent for tuberculosis culture in 234/352 (66%) patients, with 69 patients culturing *M.tb*. Of these 69 patients, 4 (6%) did not have drug susceptibility testing performed.

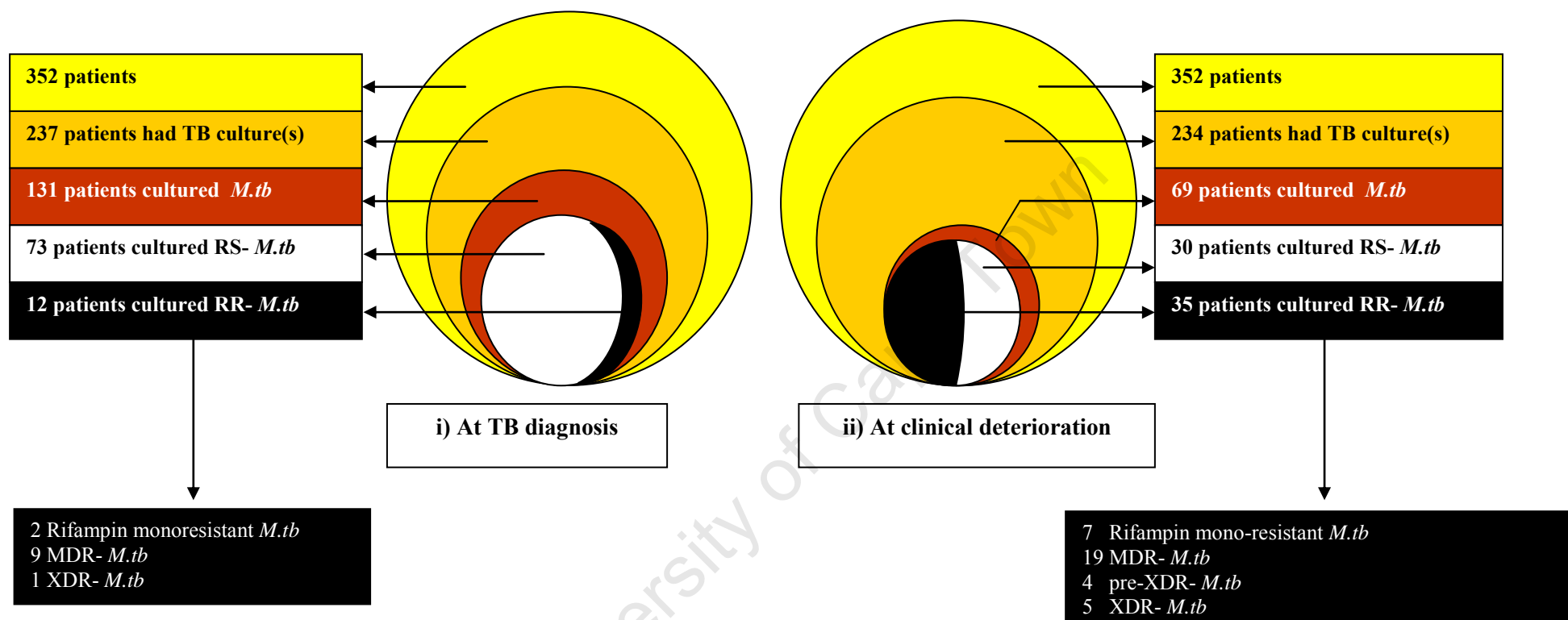


Figure 3.2: Comparison of TB cultures sent (i) at TB diagnosis and (ii) when deteriorated on antituberculosis treatment (9 January – 8 April 2007):

TB= Tuberculosis, *M.tb* = *M. tuberculosis*, RS= rifampin sensitive, RR= rifampin resistant

6 of 12 patients who had rifampin resistant *M. tuberculosis* at initial tuberculosis diagnosis, re-cultured rifampin resistant *M. tuberculosis* at clinical deterioration

i.e. 29 new cases of rifampin resistant *M. tuberculosis* were diagnosed at clinical deterioration, 8 of 73 patients who had rifampin sensitive *M. tuberculosis* at initial tuberculosis diagnosis cultured rifampin resistant *M. tuberculosis* at clinical deterioration

Of 65 patients who had antituberculosis drug sensitivity testing at clinical deterioration, 35 patients (54%) cultured rifampin-resistant *M.tb* and 30 patients (46%) cultured rifampin-sensitive *M.tb*. Thus, at clinical deterioration, 29 new cases of rifampin-resistant tuberculosis were diagnosed. These 29 cases had the following tuberculosis results at initial diagnosis: 6 *M.tb* sensitive to rifampin and isoniazid, 4 *M.tb* no sensitivities requested, and 3 no mycobacteria cultured. Sixteen of these 29 new cases of rifampin-resistant tuberculosis had no tuberculosis culture sent at initial tuberculosis diagnosis. Of 73 patients who had rifampin-sensitive *M.tb* at initial tuberculosis diagnosis, eight (11%) cultured rifampin-resistant *M.tb* at clinical deterioration. Of these eight, 2 patients cultured rifampin-mono-resistant *M.tb*, 5 MDR-*M.tb* and 1 XDR-*M.tb*, 12 to 419 days following the start of antituberculosis treatment. Thirty-seven percent of patients with rifampin-resistant *M.tb* (15/41) are known to have died within six months of initial assessment at the hospital. The median duration from assessment to death was 28 days (IQR 11–72). Forty-one patients who at initial tuberculosis diagnosis were culture negative (n= 11) or no culture was sent (n= 30), subsequently cultured *M.tb* at deterioration. In 11% (39/352) of cases, no tuberculosis cultures were sent at initial tuberculosis diagnosis and at clinical deterioration.

Extended spectrum beta lactamase producing organisms/ methicillin-resistant S. aureus.

At clinical deterioration, 214/352 (61%) tuberculosis patients had specimens sent for bacterial culture (blood cultures performed in 128 patients). Of 214 tuberculosis patients who had ≥ 1 specimen/s sent for bacterial culture, 35 (16%) cultured clinically significant organisms. Cultured organisms and specimen sites are detailed in table 3.2. Of 53 HIV-1 infected tuberculosis patients treated empirically for bacterial infections (Figure 3.1), 32 had confirmatory cultures. Twelve of these tuberculosis patients cultured either MRSA or ESBL producing organisms.

Table 3.2. Organisms cultured and sites from which they were obtained*

<i>Organisms cultured</i>	<i>n</i>	<i>Site of cultured organisms</i>	<i>n</i>
<i>Escherichia coli</i>	17	Blood	13
<i>Klebsiella</i> spp	5	Urine	10
<i>Staphylococcus aureus</i>	8	Soft tissue abscess	9
<i>Proteus</i> spp	3	Sputum	3
<i>Acinetobacter</i> spp	3	Ascitic fluid	2
<i>Enterococcus</i> spp	2	Faeces	1
<i>Enterobacter</i> spp	1		
<i>Haemophilus influenzae</i>	1		
<i>Streptococcus anginosus</i>	1		
<i>Streptococcus pneumoniae</i>	1		
<i>Pseudomonas</i> spp	1		
<i>Salmonella</i> type C	1		
*44 organisms were cultured from 38 sites from 35 patients: 3 patients cultured MRSA from ≥ 2 sites, 5 patients cultured 2 clinically significant bacteria from a single site			

(Table 3.3). The CD4+ count of these 12 tuberculosis patients was 57 cells/mm³ (median, IQR 21–80). Of nine tuberculosis patients culturing ESBL organisms, five had intermediate or high level resistance to amikacin while seven had high level resistance to ciprofloxacin. All were sensitive to imipenem, meropenem or piperacillin-tazobactam. Four tuberculosis patients had a septic illness due to MRSA, all were sensitive to vancomycin. One tuberculosis patient cultured both ESBL and MRSA organisms.

Antibiotics administered to these 12 tuberculosis patients are shown in Table 3.3. Of these 13 drug-resistant isolates, nine (69%) were drug-resistant bacteria cultured ≥ 48 hours after admission at GFJH (range 2–21 days) while four (31%) were cultured on the day of admission. Six of the twelve (50%) tuberculosis patients were admitted to hospital in the month preceding the enrolment admission – for a median duration of 10 days (IQR 8–26). Nine tuberculosis patients (75%) with ESBL/MRSA infections died within 10.5 days of obtaining the specimen (median, IQR 6.3–14.5).

TB-IRIS. We diagnosed fifty one (18%) of 291 HIV-1 infected tuberculosis patients with TB-IRIS. The median CD4+ count nadir was 65 cells/ μ L (IQR 33–113). The interval from initiation of antituberculosis treatment to initiation of ART was 69 days (median, IQR 35–94), and the interval from ART initiation to onset of TB-IRIS symptoms was 14 days (median, IQR 7–24).

Alternate illness to tuberculosis. We found an alternate illness to tuberculosis in 9% of patients (16/181) who started empiric antituberculosis treatment. In the HIV-1 infected group (n =12), alternate illnesses were predominantly new AIDS-defining illnesses which included

Table 3.3: Profile of patients with ESBL and MRSA organisms

Case	Organism cultured	Site of specimen	Susceptibility to amikacin (A)/ ciprofloxacin(C)/ vancomycin (V)			Antibiotic received	CD4+* (cells/mm ³)	ART	Outcome of admission	Duration from obtaining specimen to death (days)	Specimen obtained >48 hrs after admission	Admitted to hospital in previous 30 days	Duration of previous hospital admission (days)
			A	C	V								
1	<i>Klebsiella spp.</i> (ESBL)	Blood	R	R	-	Ceftriaxone + ciprofloxacin	4	Yes	Died	5	Yes	No	-
2	<i>Klebsiella spp.</i> (ESBL)	Blood	I	R	-	-	89	No	Died	22	Yes	Yes	5
3	<i>Klebsiella spp.</i> (ESBL)	Blood	I	R	-	Ceftriaxone	77	Yes	Died	1	Yes	Yes	72
	MRSA	Pus swab x2	-	-	S					13	No		
4	<i>E. coli</i> (ESBL)	Blood	I	R	-	Amikacin	20	Yes	Died	61	Yes	Yes	11
5	<i>E. coli</i> (ESBL)	Blood	S	R	-	Amikacin	51	No	Died	6	Yes	Yes	9
6	<i>E. coli</i> (ESBL)	Blood	S	R	-	Amikacin	76	No	Discharged alive	-	Yes	No	-
7	<i>E. coli</i> (ESBL)	Midstream urine	S	R	-	Amikacin	unknown	Yes	Discharged alive	-	Yes	No	-
8	<i>E. coli</i> (ESBL)	Blood	R	S	-	-	167	Yes	Died	10	No	Yes	30
9	<i>E. coli</i> (ESBL)	Blood	S	S	-	Amikacin	21	No	Died	11	No	No	-
10	MRSA	Pus swab x2	-	-	S	Cloxacillin	38	No	Died	10	Yes	No	-
11	MRSA	Blood	-	-	S	Vancomycin	166	No	Died	8	Yes	No	-
12	MRSA	Pus swab x2	-	-	S	Clindamycin	62	Yes	Discharged alive	-	No	Yes	7
Median (IQR)							57 (21-80)			10 (8 – 14)			
Percentage								50%	Died 75%		Yes, 69%	Yes, 54%	

Klebsiella spp. (ESBL)=*Klebsiella spp.* demonstrating extended spectrum beta-lactamase activity, sensitive to Imipenem, Meropenem or Piperacillin-tazobactam, *E.coli* (ESBL)=*Escherichia coli* demonstrating extended spectrum beta-lactamase activity, sensitive to Imipenem, Meropenem or Piperacillin-tazobactam, MRSA = methicillin-resistant *Staph. aureus*, resistant to cloxacillin, Susceptibility: R= Resistant, I= Intermediate resistance, S= sensitive, ART = antiretroviral therapy, * = all 12 patients were HIV-1-infected

Kaposi's sarcoma (n= 2), *Pneumocystis jiroveci* pneumonia (n =2) and non-Hodgkin's lymphoma (n =2).

Inpatient mortality

Inpatient mortality did not differ significantly according to HIV-1 status ($p= 0.566$, Table 3.1). Inpatient deaths of HIV-1 infected patients (n = 43) were mainly due to bacterial infections (n= 12), new AIDS-defining illnesses (n =10), enteric illnesses (n= 8), and pulmonary embolism (n= 5).

Incidence of clinical deterioration

An estimated 3500 patients start antituberculosis treatment every three months at public sector primary care tuberculosis clinics within our catchment area (Judy Caldwell, Western Cape Tuberculosis Control Programme- personal communication). Private sector antituberculosis treatment is not provided within our catchment area. During the 3-month study period, 352 patients (10%, 95% confidence interval: 9–11%) deteriorated during antituberculosis treatment and required referral to our facility.

3.3 Discussion

We undertook this study to investigate which reasons for clinical deterioration during antituberculosis treatment were most important in a setting with a high prevalence of tuberculosis and HIV-1. Focusing on this particular patient group may inform strategies seeking to improve the performance of tuberculosis and HIV-1 programmes, as well as optimise the rational use of limited resources. We found that at least 10% of patients starting antituberculosis treatment at the tuberculosis clinics experienced clinical deterioration and require referral to hospital. We also found that drug resistant *M.tb* and drug resistant bacterial infections were important reasons for clinical deterioration and death. Additional illnesses to tuberculosis accounted for most referrals, particularly new AIDS-defining illnesses and bacterial infections.

Our findings are best explained in the context of rigorous admission criteria due to bed pressures at our 200-bed referral hospital; only 7.5% of adult patients referred to the emergency department are admitted to the medical wards. Admission is prioritised for life-threatening illnesses that require intravenous fluids or intravenous antibiotics (such as antibacterial or antifungal agents), drug resistant *M.tb* requiring daily inpatient intramuscular amikacin or kanamycin injections (while awaiting a bed at the nearby MDR-TB hospital) or patients requiring monitored supervision of antituberculosis treatment because of severe disease. It is inevitable that a larger group of patients with clinical deterioration not meeting such strict admission criteria was not referred to hospital for assessment. Chapter 4 reports our subsequent prospective study. We found that this larger group existed and that the causes for clinical deterioration were similar (chapter 7).

Nearly one-sixth of our patients cultured either drug resistant *M.tb* or other drug resistant bacterial infections. This has two important implications. Firstly we have identified a clinical subgroup of patients, namely patients deteriorating during antituberculosis treatment, in whom the incidence of rifampin resistant tuberculosis (8.2%, 95% confidence interval: 5–11%) is high compared to the reported percentage of 2.5% for tuberculosis cases in South Africa (Weyer 2007). Secondly, tuberculosis and HIV-1 co-infected patients in a hospital setting appear prone to acquire ESBL and MRSA organisms. Studies need to be conducted at other hospitals to confirm our findings.

Nosocomial acquisition of XDR- *M.tb* among HIV-1 infected patients with a very poor outcome has recently been described in South Africa (Gandhi 2006); the contribution to death by co-morbid HIV-1 associated illnesses, however, was not discussed in that report. Our study suggests co-morbid HIV-1 associated illnesses may play a role in the death of such patients (Figure 3.1). The evolution from isoniazid and rifampin sensitive *M.tb* to rifampin mono-resistant-, MDR-, preXDR- and XDR- *M.tb* in eight patients may be due to initial mixed *M.tb* strain infection (Niemann 2000), exogenous re-infection with rifampin resistant *M.tb* (Small 1993) or the rapid development of *M.tb* drug resistance mutations despite a multidrug-regimen i.e. amplified drug resistance. Nosocomial re-infection of HIV-1 infected patients with MDR- and XDR *M. tb* has been documented in both resource rich and resource-constrained settings (Small 1993, Gandhi 2006). The possibility that drug resistance evolved rapidly in drug susceptible cases receiving optimal treatment appears less likely – based on clinical history and collateral information regarding adherence obtained from tuberculosis clinics and relatives. The decline in proportion of patients culturing *M.tb* at initial tuberculosis diagnosis and subsequent clinical deterioration may indicate efficacious antituberculosis treatment, inability of the patient to expectorate sputa for culture, or difficulty obtaining a non-pulmonary specimen for culture.

Nosocomial acquisition of drug resistant bacteria is also suggested by the temporal association between i) the timing of specimens that cultured ESBL and MRSA organisms and ii) the duration of current and previous hospital admissions. The unavailability of appropriate antimicrobial agents likely contributed to the high mortality rate in these 12 HIV-1 infected tuberculosis patients. At the time of the study, the standard regimen for suspected nosocomial sepsis was amikacin and ceftriaxone in the absence of renal impairment, and ciprofloxacin and ceftriaxone if present. Vancomycin was available to treat MRSA. Because of our study findings, ertapenem is now available.

The incidence of additional illnesses such as new AIDS-defining illnesses, bacterial infections and gastroenteritis is indicative of the profound immune suppression in the HIV-1 infected group. Multiple opportunistic infections occur simultaneously in AIDS patients. A necropsy study of HIV-1 infected patients from Brazil reported more than one post-mortem diagnosis in 52% of the patients. In 48% of post-mortems, at least one AIDS-related disease not clinically suspected (Borges 1997). These researchers recommended aggressive investigation for infections and cancers in sick patients with AIDS, particularly in those not responding to initial antimicrobial therapy (Borges 1997). Bacterial infections and enteric illnesses were found in 26% and 18% of HIV-1 infected patients, respectively, in our study. Current provincial government protocols recommend starting co-trimoxazole prophylaxis in all HIV-1 positive patients one month after initiating antituberculosis treatment in order to differentiate between side effects from antituberculosis treatment and co-trimoxazole (SATB guidelines 2004, TB/HIV Policy 2004). This would likely reduce bacterial infections. Adherence to this recommendation was not assessed in this study but was assessed in the subsequent study

(chapter 4). Although only 48% of patients eligible for ART were receiving ART at deterioration, it is likely that some patients subsequently initiated ART.

TB-IRIS was a final diagnosis in 18% (51/291) of HIV-1 infected patients. This probably reflects the high incidence of disseminated tuberculosis and the relatively late initiation of ART in profoundly immune suppressed HIV-1 patients. TB-IRIS is more likely to occur in patients with a low baseline CD4+ count, disseminated tuberculosis, and a short interval from initiation of antituberculosis treatment to ART (Breton 2004, Shelburne 2006, Dhasmana 2008).

Fifteen cases of venous thrombo-embolic disease (12 deep vein thrombosis and 3 pulmonary embolus) were observed among this cohort. HIV-1 and rifampin are postulated risk factors for venous thrombo-embolic disease (White 1989, Matta 2008).

Both HIV-1 uninfected and infected patients had prolonged admissions (9.5 days) compared to the typical duration of admission at GFJH (4 days) (Burch 2006). Longer inpatient admissions increase the risk of acquisition of nosocomial drug resistant pathogens, particularly in immune-compromised patients.

Our study's limitations fundamentally relate to its design within routine care in an exceptionally busy setting. Firstly, studies based in hospitals suffer referral bias. Therefore, the extent of the problem of clinical deterioration during antituberculosis treatment cannot be precisely determined. This problem is clearly very significant, and is likely to impact adversely on overall national tuberculosis programme success. Secondly, the initial tuberculosis diagnosis was often defined by clinical algorithm rather than mycobacterial culture and, even at clinical deterioration, not all patients were sampled. Of all 352 tuberculosis patients assessed at

deterioration, 182 (52%) did not culture *M.tb* at both tuberculosis diagnosis and at deterioration. Thirdly, final diagnosis relied on available diagnostic modalities, better than in many parts of Africa but not state-of-the-art. Resistance to second line antituberculosis agents was not always assayed: thus our estimates of pre-XDR and XDR- *M.tb* may be falsely low. Genotyping of drug resistance *M.tb* and other bacterial strains would have allowed us to better assess the likelihood of nosocomial transmission. 8% of patients were not tested for HIV-1 infection. All these factors were considered in a follow-up clinic-based study of this problem reported in chapter 4.

As a result of these findings, basic infection control measures have been strengthened; extraction fans have been installed at high congestion areas and natural ventilation is encouraged to reduce *M.tb* transmission. N95 respirator masks are readily available to patients, relatives and health care workers to reduce aerosol transmission and infection of tuberculosis. In September 2009, 3 isolation beds were installed into the female medical ward. Currently, the male ward is undergoing similar architectural modifications to further improve ventilation and reduce the risk of *M.tb* transmission.

University of Cape Town

Chapter 4

Clinical Deterioration during Antituberculosis Treatment in South Africa: Incidence, Causes and Risk Factors

University of Cape Town

Contributions: The study was conceived by the candidate, Robert J. Wilkinson and Graeme Meintjes. The study was designed by the candidate, Suzaan Marais, Robert J. Wilkinson, Gary Maartens, Helen McIlleron, Virginia De Azevedo, Helen Cox, Cheryl McDermid and Graeme Meintjes. The data was obtained by the candidate, Suzaan Marais, Simiso Sokhela, Janisha Patel and Graeme Meintjes. The data was interpreted by the candidate, Suzaan Marais, Robert J. Wilkinson, Feriyl Bhaijee, Gary Maartens, Helen McIlleron, Virginia De Azevedo, Helen Cox, Cheryl McDermid, Simiso Sokhela, Janisha Patel and Graeme Meintjes. The candidate prepared the first draft, which was critically revised by Suzaan Marais, Robert J. Wilkinson, Feriyl Bhaijee, Gary Maartens, Helen McIlleron, Virginia De Azevedo, Helen Cox, Cheryl McDermid, Simiso Sokhela, Janisha Patel and Graeme Meintjes.

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Our preliminary study informed us that clinical deterioration was a significant problem at our referral hospital, accounting for 17% of inpatient medical admissions. Moreover, drug resistant bacteria and AIDS-defining illnesses were important causes for deterioration and death.

However, we still did not know the incidence or risk factors for clinical deterioration. Also, we did not know if the causes for clinical deterioration differed between the referral hospital and tuberculosis clinic.

In this subsequent study, we assessed patients at initiation of antituberculosis treatment and followed them for 24 weeks, in order to determine the incidence, causes and risk factors for

clinical deterioration. We also discuss initiatives to reduce the high burden of clinical deterioration in resource-limited settings.

4.1 Methods

We conducted a prospective cohort study at Khayelitsha Site B tuberculosis clinic (Cape Town, South Africa) from 1 June 2008 through 15 February 2009. We assessed adult (≥ 18 years age) patients diagnosed with tuberculosis at Khayelitsha Site B tuberculosis clinic from 1 June 2008 through 31 August 2008 (3-month assessment period). Patients were followed for 24 weeks from initiation of antituberculosis treatment. Our study was nested within a tuberculosis drug susceptibility testing (DST) survey in which first-line DST (for isoniazid and rifampin) was routinely performed, regardless of HIV-1 status or previous tuberculosis. The Research Ethics Committee of the University of Cape Town approved this study (REC 178/2008).

Study site and setting. Khayelitsha Site B tuberculosis clinic is a primary-level outpatient health care facility, which serves ~100,000 indigent people within its catchment area. Approximately 1,200 adult (≥ 18 years age) cases of tuberculosis are diagnosed per annum at Site B tuberculosis clinic. HIV-1 voluntary counselling and testing is offered to all patients diagnosed with tuberculosis at Site B Khayelitsha. In 2006, the antenatal HIV-1 seroprevalence in this community was 33% (95%: 29.1 – 36.9%) (Provincial Administration of the Western Cape [PAWC] 2007). As of March 2009, >5,000 people had initiated ART at Khayelitsha Site B HIV-1 clinic. The national protocols for treating tuberculosis and HIV-AIDS were described in Chapter 2.

Definitions. “Tuberculosis patients” refers to patients diagnosed with tuberculosis and initiated on antituberculosis treatment. We defined microbiologically-confirmed tuberculosis as

Mycobacterium tuberculosis cultured or acid-fast bacilli visualised in a biological specimen. Biological specimens included sputum, pleural fluid, urine, nodal aspirates, pericardial aspirates and cerebrospinal fluid. We defined microbiologically-unconfirmed tuberculosis according to the WHO case definitions for smear-negative and extra-pulmonary tuberculosis (WHO 2007). Biological specimens were obtained at tuberculosis diagnosis, at 8 and 20 weeks' follow-up and at clinical deterioration. First-line DST using the GenoType MTBDRplus assay (Hain Lifescience 2009) was performed on biological specimens that were smear-positive for acid-fast bacilli and/ or culture positive for *M.tb*. We defined multidrug-resistant (MDR) tuberculosis as *M.tb* with resistance to isoniazid and rifampin.

We defined clinical deterioration as symptomatic worsening or failure to stabilise within 24 weeks following initiation of antituberculosis treatment. We subdivided the causes of clinical deterioration into HIV-1 related illnesses and HIV-1 unrelated illnesses. HIV-1 related causes included AIDS-defining illnesses (according to WHO criteria), and non AIDS-defining illnesses i.e. Non AIDS-defining HIV-1 related infections. HIV-1 unrelated illnesses included tuberculosis-related illnesses, and non tuberculosis-related illnesses i.e. co-morbid illnesses. Tuberculosis-related illnesses included MDR-TB, deterioration due to poor adherence, paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) and paradoxical reactions. Co-morbid illnesses were acute illnesses that occurred after tuberculosis diagnosis and were considered not to be directly attributable to HIV-1 or tuberculosis. Pre-existing conditions were not included in co-morbid illnesses.

We defined TB-IRIS according to the consensus clinical case definition for resource-limited settings (Meintjes 2008). We defined paradoxical reactions using an adaptation of this

consensus clinical case definition of paradoxical TB-IRIS (Meintjes 2008). Three criteria were required for a diagnosis of paradoxical reaction:

1. Diagnosis of tuberculosis (microbiologic confirmation or according to WHO criteria [WHO 2007]) with initial response to antituberculosis treatment,
2. The recurrence/ new onset of tuberculosis disease manifestations within 24 weeks of antituberculosis treatment, and
3. Exclusion of alternative explanations for clinical deterioration (such as antituberculosis drug resistance, poor adherence, drug toxicity or reaction, or an additional infection).

Paradoxical reactions were diagnosed in both HIV-1 infected and uninfected patients. HIV-1 infected patients receiving ART at clinical deterioration were diagnosed with TB-IRIS rather than a paradoxical reaction, according to our definitions.

We defined a patient as being lost to follow-up if we were unable to trace a patient 24 weeks after initiation of antituberculosis treatment. We used clinic and hospital medical notes, as well as the National Health Laboratories Service database to trace specimens and the Provincial Government of the Western Cape's electronic tuberculosis register to trace patients.

Assessment of patients with clinical deterioration. The study was discussed with physicians and nurses at the tuberculosis clinic prior to commencement of the study. Adult patients with clinical deterioration within 24 weeks of initiation of antituberculosis treatment were prospectively evaluated (regardless of severity of illness, eventual diagnosis, or inpatient or outpatient management) to determine the reason for clinical deterioration. Stable patients were assessed at Khayelitsha Site B tuberculosis clinic; patients requiring hospital admission or invasive outpatient procedures were assessed at GF Jooste Hospital (the clinic's referral hospital). At clinical deterioration, we routinely obtained biological specimens to investigate

drug-resistant *M. tuberculosis*. Specimens were also cultured for bacterial organisms. Deaths were recorded using the Provincial Government of the Western Cape's electronic tuberculosis register and Clinicom Systems, and included both inpatient deaths and death outside the hospital. Various spellings and combinations of a patient's first and last name were utilised, with the date of birth, to follow-up patients. Where patients were alive or lost to follow-up at 24 weeks, the electronic database was checked on three successive occasions, each 2 weeks apart.

Data collection and analysis. Data collected included demographic information, tuberculosis specimen results, HIV-1 status, ART, CD4+ cell count at tuberculosis diagnosis, diagnosis at clinical deterioration and outcome 24 weeks after initiation of antituberculosis treatment. Statistical analyses were performed using Stata 10.0 (Texas, USA). Wilcoxon rank-sum and Kruskal-Wallis tests were used for group comparisons, and Fisher's exact tests to compare proportions. Variables with the outcome of interest were entered into Cox proportional hazards models to assess independent effects of covariates. Significant variables were removed from the model to assess whether these effects remained. The assumptions of the Cox model were verified; non-informative censoring was performed and the test for the proportional hazards assumption was not significant. We censored patients at clinical deterioration when performing Cox-proportional hazards model analysis and relative risk calculations. We censored patients at loss to follow-up when determining the incidence rate of clinical deterioration (illnesses diagnosed per 100 months of follow-up).

4.2 Results

During the 3-month assessment period (figure 4.1), 305 adults (≥ 18 years age) initiated antituberculosis treatment, 7 of whom had untraceable clinical records and 6 of whom declined HIV-1 testing. We restricted our data analysis to the 292 (96%) patients whose HIV-1 status and clinic records were available. In 209 HIV-1 infected and 83 HIV-1 uninfected patients, loss to follow-up (46 [22%] of 209 vs. 29 [35%] of 83, p -value= 0.026) and mortality (16 [8%] of 209 vs. 1 [1%] of 83, p -value= 0.048) differed significantly at 24 weeks of follow-up.

At initial tuberculosis diagnosis (table 4.1), HIV-1 infected patients were more likely than HIV-1 uninfected patients to be female, be of younger age, have extra-pulmonary tuberculosis and be diagnosed with tuberculosis at the referral hospital. HIV-1 uninfected patients were more likely than HIV-1 infected patients to have microbiologic confirmation of tuberculosis at initial tuberculosis diagnosis and during the 24 weeks of follow-up.

Prior to tuberculosis diagnosis, 34 (23%) of 145 HIV-1 infected patients qualifying for ART under the national guidelines were receiving ART. Six months later, 109 (75%) of 145 patients had received ART.

Causes of Clinical Deterioration and Hospital Admission

During the 24 weeks of follow-up, 117 (40%, 95% CI: 35 –46%) of 292 tuberculosis patients experienced clinical deterioration, of whom 101 were HIV-1 infected and 16 were HIV-1 uninfected. Causes of clinical deterioration (table 4.2) included: co-morbid illnesses (70 patients), tuberculosis-related illnesses (47 patients), non AIDS-defining HIV-1 related infections (25 patients) and AIDS-defining illnesses (21 patients). Peripheral neuropathy,

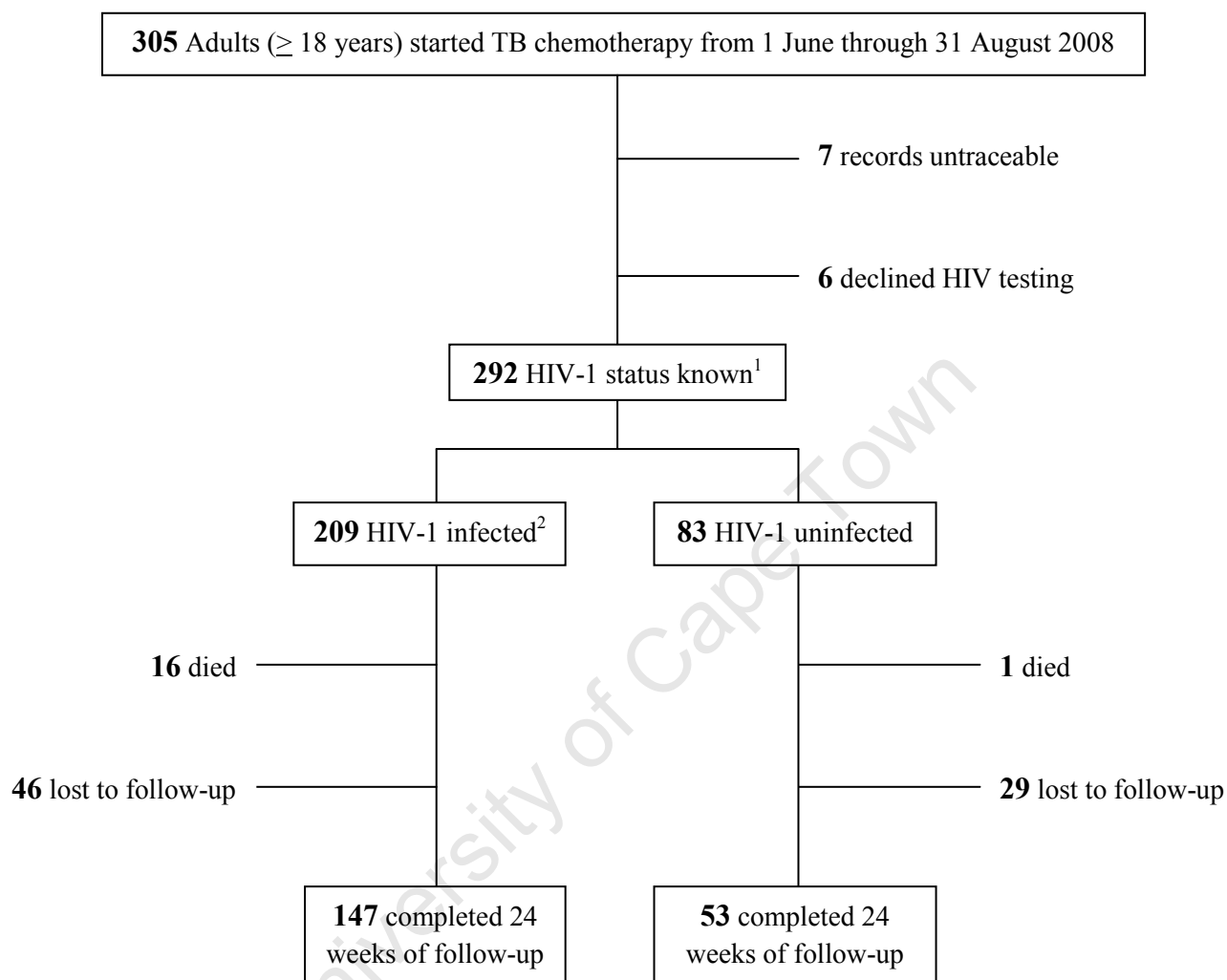


Figure 4.1: Flow-diagram of 305 adult patients who started antituberculosis treatment during 3-month assessment period (1 June – 31 August 2008)

¹ Subsequent data analysis for 292 patients with known HIV-1 status

² CD4+ counts not performed in 3 of 209 HIV-1 infected patients

Table 4.1: Baseline characteristics and microbiologic confirmation of tuberculosis in 209 HIV-1 infected and 83 HIV-1 uninfected patients receiving antituberculosis treatment

	HIV-1 infected (n=209)	HIV-1 uninfected (n=83)	p-value
Female, n (%)	112 (54)	25 (30)	<0.001
Age in years, median (IQR)	35 (30-41)	38 (29-49)	0.035
CD4+ count at TB diagnosis, n (%):			
>350 cells/mm ³	36 (17)	-	
200 – 350 cells/mm ³	37 (18)	-	
< 200 cells/mm ³	133 (64)	-	
Not performed	3 (1)	-	
Previous TB, n (%)	71 (34)	19 (23)	0.070
TB diagnosed at referral hospital, n (%):	79 (38)	13 (16)	<0.001
Microbiologic confirmation at TB diagnosis¹, n (%)	123 (59)	68 (82)	<0.001
Drug susceptibilities known at TB diagnosis¹, n (%)	67 (32)	49 (59)	<0.001
Culture sent at TB diagnosis, n (%)	169 (81)	68 (82)	1.000
Extra-pulmonary TB, n (%):	82 (39)	14 (17)	<0.001
Weight at TB diagnosis in kg, median (IQR)	54 (49–62)	54 (49–64)	0.644
Microbiologic confirmation at TB diagnosis and during 24 weeks of follow-up², n (%)	153 (73)	70 (84)	0.048
Drug susceptibilities known at TB diagnosis and during 24 weeks of follow-up², n (%)	117 (56)	57 (69)	0.296
Culture sent at TB diagnosis and/ or during 24 weeks of follow-up, n (%)	190 (91)	76 (92)	1.000
¹ within 14 days of TB diagnosis. No TB specimens sent in 12 HIV-1 infected patients and 3 HIV-1 uninfected patients at TB diagnosis.			
² no TB specimens sent in 2 HIV-1 infected patients.			
TB = tuberculosis, IQR = inter-quartile range, OR = odds-ratio, 95% CI = 95% confidence interval			

Table 4.2: Illnesses (n=199) in 101 HIV-1 infected and 16 HIV-1 uninfected patients who experienced clinical deterioration during 24 weeks of antituberculosis treatment

	HIV-1 infected	HIV-1 uninfected
number of illnesses = n, (total number of patients = pnts)	180 (101)	19 (16)
1. Tuberculosis-related illnesses, n (pnts)	49 (43)	5 (5)
TB-IRIS	22	-
Paradoxical reaction	17	4
MDR-TB ¹	4	1
Deterioration due to poor adherence	5	-
Low rifampin concentration in comparison to the recommended range	1	-
2. AIDS-defining illnesses, n (pnts)	29 (21)	-
Oesophageal candida	11	-
<i>Pneumocystis jiroveci</i> pneumonia ²	5	-
Cryptococcal meningitis	4	-
Cytomegalovirus retinitis	3	-
Other ³	6	-
3. Non-AIDS defining HIV-1 related infections, n (pnts)	27 (25)	-
Superficial herpes infection ⁴	9	-
Bacterial infection ⁵	9	-
Fungal (oral/ vaginal/superficial) infection	9	-
4. Co-morbid illnesses, n (pnts)	76 (59)⁶	14 (11)⁷

Key:

TB = tuberculosis; TB-IRIS = TB-associated immune reconstitution inflammatory syndrome

¹ MDR-TB: *Mycobacterium tuberculosis* resistant to rifampin and isoniazid

² All patients diagnosed with *Pneumocystis jirovecii* pneumonia had a history of good compliance with trimethoprim sulfamethoxazole chemoprophylaxis (160/800mg daily).

³ Other = HIV-1 associated nephropathy (2), HIV-1 associated encephalopathy (2), Disseminated Kaposi's sarcoma (2)

⁴ 5 patients diagnosed with herpes simplex, 4 patients diagnosed with herpes zoster (all < 1 month duration)

⁵ Urinary tract infection (3) – *E.coli*, *Klebsiella sp.*, enterococcus; Pelvic inflammatory disease (3); *H.influenza* pneumonia (1); otitis media(1); carbuncle(1)

⁶ **76 illnesses diagnosed in 59 patients:**

CD4+ > 350 cells/ mm³ = 7 illnesses diagnosed in 7 patients: deep venous thrombosis (2); sacroiliitis (1); acute asthmatic attack (1); cardiomyopathy (1); severe epistaxis (1); supra-condylar fracture (1),

CD4+ 200 – 350 cells/ mm³ = 12 illnesses diagnosed in 7 patients: enteric illness with no pathogen isolated (4); hyperglycaemic emergency (2); seizures, cause not determined (2); acute asthmatic attack (1); efavirenz side-effect (1); deep venous thrombosis (1); haemorrhoids/ fistula-in-ano (1),

CD4+ < 200 cells/mm³ = 56 illnesses in 44 patients: peripheral neuropathy (12); enteric illness with no pathogen isolated (8); deep venous thrombosis (7); dermatitis (4); cerebrovascular accident (3); scabies (3); minor traumatic injury (3); pneumothorax (2); pancreatitis (1); cardiomyopathy (1); miscarriage (1); cryptococcal IRIS (immune reconstitution inflammatory syndrome) adenitis (1); drug induced hepatitis (1); cause not determined but patient subsequently improved (9).

⁷ **14 illnesses diagnosed in 11 patients:** Drug side effect (3) – hydrochlorothiazide (1), pyrazinamide (1), kanamycin (1); hyperglycaemic emergency (2); post-TB bronchiectasis (1); peripheral neuropathy (1); pulmonary silicosis (1); miscarriage (1); cause not determined but patient subsequently improved (5)

enteric illness and deep venous thrombosis were frequent reasons for co-morbid illnesses. TB-IRIS and paradoxical reactions were frequent reasons for tuberculosis-related illnesses. Oesophageal candida, *Pneumocystis jirovecii* pneumonia and cryptococcal meningitis were frequent reasons for AIDS-defining illnesses. Of 117 patients who experienced deterioration, 30 (26%) required hospital admission [27 (27%) of 101 HIV-1 infected and 3 (19%) of 16 HIV-1 uninfected patients (p-value= 0.756)]. Causes of inpatient hospital admission were paradoxical reaction or TB-IRIS (9 patients), new AIDS-defining illness (8 patients), deep venous thrombosis (6 patients), MDR-TB (2 patients), cardiomyopathy (1 patient), pneumothorax (1 patient), symptomatic deterioration due to poor adherence with antituberculosis treatment (1 patient), hyperglycaemic emergency (1 patient) and seizure disorder (1 patient).

Risk Factors for Clinical Deterioration

In the 292 tuberculosis patients (Table 4.3a), 4 factors were significantly associated with clinical deterioration in univariate analysis: HIV-1 infection, diagnosis of tuberculosis at the referral hospital, evidence of extra-pulmonary tuberculosis, and absence of a DST result at tuberculosis diagnosis. Only HIV-1 infection (figure 4.2a) remained significant in multivariate analysis (hazard ratio [HR]= 2.0, 95% CI=1.1–3.6).

In subsequent analysis (figures not shown), we assessed whether the probability of clinical deterioration from non HIV-1 related causes was associated with HIV-1 infection. In univariate analysis, we found a significant association with HIV-1 infection and non HIV-1 related causes for deterioration (RR = 1.3, 95% CI: 1.07 – 1.50). However, in the Cox proportional hazards model, this significant association was not confirmed (HR = 1.5, 95% CI: 0.81-2.64). This

analysis suggests that HIV-1 infection is a significant variable for clinical deterioration because of HIV-1 related illnesses (either AIDS- or non AIDS-defining illnesses).

In the 209 HIV-1 infected tuberculosis patients (Table 4.3b), 3 factors were significantly associated with clinical deterioration in univariate analysis: a lower CD4+ count, diagnosis of tuberculosis at the referral hospital, and ART received during antituberculosis treatment. Only a lower CD4+ stratum at tuberculosis diagnosis (figure 4.2b) remained significant in multivariate analysis (HR = 1.5, 95% CI=1.1–2.2).

In subsequent analysis (figures not shown), we assessed whether the probability of clinical deterioration from non HIV-1 related causes was associated with decreasing CD4+ counts. We found no significant association with decreasing CD4+ counts and non HIV-1 related causes in both univariate analysis ($P=0.189$) and the Cox proportional hazards model (HR=1.3, 95%CI: 0.88 – 1.97). This analysis suggests that decreasing CD4+ counts are a significant variable for clinical deterioration because of HIV-1 related illnesses (either AIDS- or non AIDS-defining illnesses).

Table 4.3a: Univariate risk factor analysis for clinical deterioration in 292 patients who received antituberculosis (TB) treatment					
	Deterioration (n=117)	No deterioration (n=175)	p-value	RR	95% CI
Female, n (%)	62 (53)	75 (43)	0.095	1.3	0.96 - 1.69
Age in years, median (IQR)	36 (31-45)	35 (29- 43)	0.327		
Previous TB, n (%)	35 (30)	55 (31)	0.798	1.0	0.70 - 1.30
HIV-1 infected, n (%)	101 (86)	106 (61)	<0.001	2.6	1.63 - 4.12
Weight at TB diagnosis in kg, median (IQR)	54 (49-61)	54 (49-64)	0.599		
TB diagnosed at hospital, n (%):	52 (44)	40 (23)	<0.001	1.7	1.33 - 2.28
Extra-pulmonary TB, n (%):	50 (43)	46 (26)	0.005	1.5	1.16 - 2.00
Microbiologic confirmation at TB diagnosis ¹ , n (%)	69 (59)	122 (70)	0.061	0.8	0.58 - 1.00
Microbiologic confirmation with drug susceptibilities known at TB diagnosis ¹ , n (%)	37 (32)	79 (45)	0.028	0.7	0.51 - 0.96
Table 4.3b: Univariate risk factor analysis for clinical deterioration in 209 HIV-1 infected patients who received TB treatment					
	Deterioration (n=101)	No deterioration (n=108)	p-value	RR	95% CI
Female, n (%)	55 (54)	57 (53)	0.890	1.0	0.78 - 1.37
Age in years, median (IQR)	36 (29 - 43)	35 (30 - 41)	0.517		
Previous TB, n (%)	31 (31)	40 (37)	0.381	0.9	0.63 - 1.18
Median CD4 count (cells/mm ³), n (IQR)	79 (33 - 199)	184 (80 - 350)	<0.001		
Weight at TB diagnosis in kg, median (IQR)	55 (49 - 62)	53 (49 - 62)	0.885		
TB diagnosed at hospital, n (%)	50 (50)	28 (26)	<0.001	1.6	1.26 - 2.16
Extra-pulmonary TB, n (%)	46 (46)	36 (33)	0.089	1.3	0.98 - 1.71
Microbiologic confirmation at TB diagnosis ¹ , n (%)	57 (56)	66 (61)	0.574	0.9	0.68 - 1.20
Microbiologic confirmation with drug susceptibilities known at TB diagnosis ¹ , n (%)	29 (29)	38 (35)	0.374	0.9	0.62 - 1.17
TMP-SMX ² chemoprophylaxis, n (%)	91 (90)	90 (83)	0.162	1.4	0.84 - 2.36
Antiretroviral treatment ³ during TB treatment, n (%)	62 (61)	47 (44)	0.013	1.5	1.09 - 1.96
¹ Within 14 days of TB diagnosis. No TB specimens sent in 12 HIV-1 infected patients and 3 HIV-1 uninfected patients at TB diagnosis. ² TMP-SMX = trimethoprim sulfamethoxazole 160/800mg daily ³ Antiretroviral treatment regimens were as follows: D4T/3TC/EFV (89 patients), D4T/3TC/NVP (3 patients), AZT/3TC/EFV (13 patients), AZT/3TC/NVP (2 patients), TDF/3TC/EFV (2 patients); D4T= stavudine 30mg twice daily, 3TC = lamivudine 150mg twice daily, EFV = efavirenz 600mg nocte, AZT = zidovudine 300mg twice daily, NVP = nevirapine 200mg twice daily, TDF = tenofovir 300mg daily TB = Tuberculosis, RR = relative risk, 95% CI = 95% confidence interval					

Figure 4.2a: Hazard ratios (95% CI) of risk factors for clinical deterioration during 24 weeks of antituberculosis (TB) treatment in 292 patients (Cox proportional hazards model)

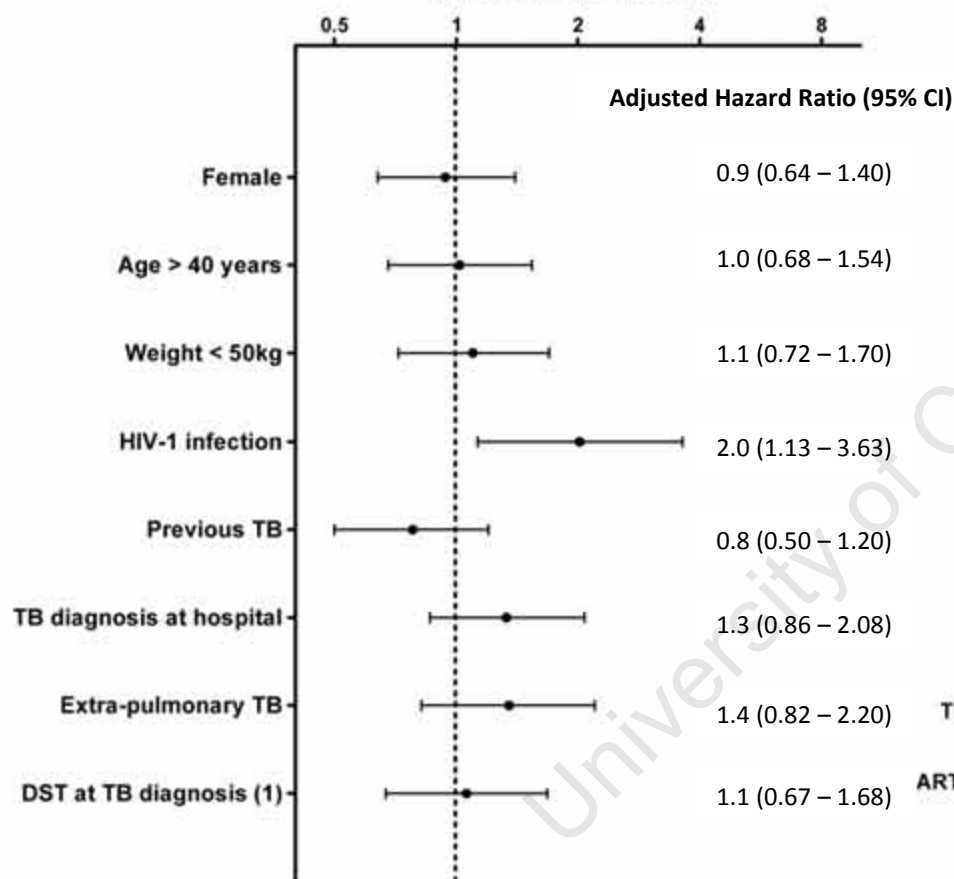
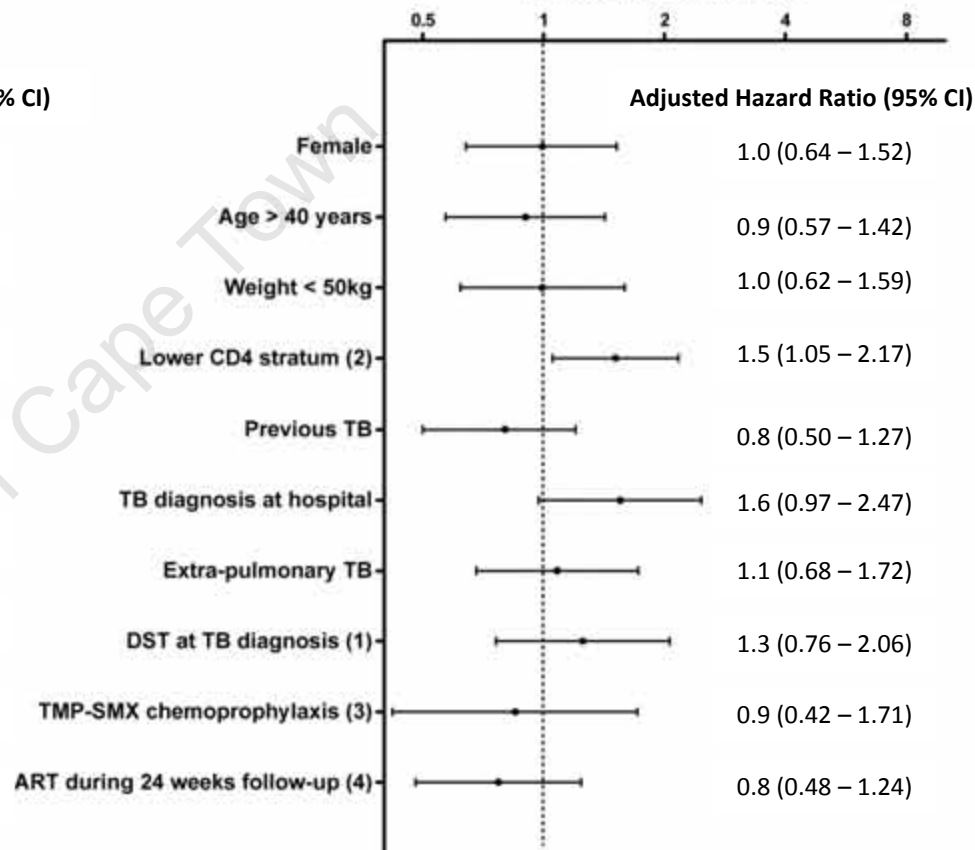


Figure 4.2b: Hazard ratios (95% CI) of risk factors for clinical deterioration during 24 weeks of TB treatment in 206 HIV-1 infected patients (Cox proportional hazards model)



*CD4 count not performed in 3 of 209 HIV-1 infected patients

(1) Microbiologic confirmation with drug susceptibilities known at tuberculosis diagnosis

(2) CD4 strata used: Stratum 1 = CD4+ > 350 cells/ μ L, stratum 2 = CD4+ from 200 – 350 cells/ μ L, stratum 3 = CD4+ < 200 cells/ μ L;

(3) TMP-SMX chemoprophylaxis = Trimethoprim sulfamethoxazole 160/800mg daily

(4) Antiretroviral treatment regimens as follows: D4T/3TC/EFV (89), D4T/3TC/NVP (3), AZT/3TC/EFV (13), AZT/3TC/NVP (2), TDF/3TC EFV (2); D4T= stavudine 30mg twice daily, 3TC = lamivudine 150mg twice daily, EFV = efavirenz 600mg nocte, AZT = zidovudine 300mg twice daily, NVP = nevirapine 200mg twice daily, TDF = tenofovir 300mg daily

TB = tuberculosis, DST = drug susceptibility testing, ART = antiretroviral treatment, 95% CI = 95% confidence interval

Relative Risk and Incidence Rate of Clinical Deterioration

HIV-1 infection and a low CD4⁺ count were the only significant risk factors for clinical deterioration in multivariate analysis. We therefore used HIV-1 status and CD4⁺ stratum to determine the relative risk and incidence rate of clinical deterioration during the 24 weeks of follow-up. Using HIV-1 uninfected patients as referent group, the relative risk (RR) of clinical deterioration increased as the CD4⁺ counts in HIV-1 infected patients decreased (CD4⁺ >350 cells/ μ L: RR = 1.4, 95% CI = 0.7–2.9; CD4⁺ 200–350 cells/ μ L: RR = 2.0, 95% CI = 1.1–3.6; CD4⁺ <200 cells/ μ L: RR = 3.0, 95% CI = 1.9–4.7). The incidence rate (IR) of clinical deterioration (illnesses diagnosed per 100 months of follow-up) also increased as the CD4⁺ counts decreased. Incidence rates differed significantly between HIV-1 uninfected patients (IR = 5.1, 95% CI = 3.1–7.5) and HIV-1 infected patients with a CD4⁺ count of 200–350 cells/ μ L (IR = 12.3, 95% CI = 8.2–17.0). Similarly, incidence rates differed significantly between HIV-1 infected patients with a CD4⁺ count of 200–350 cells/ μ L and HIV-1 infected patients with a CD4⁺ count < 200 cells/ μ L (IR = 20.7, 95% CI 17.8–23.9).

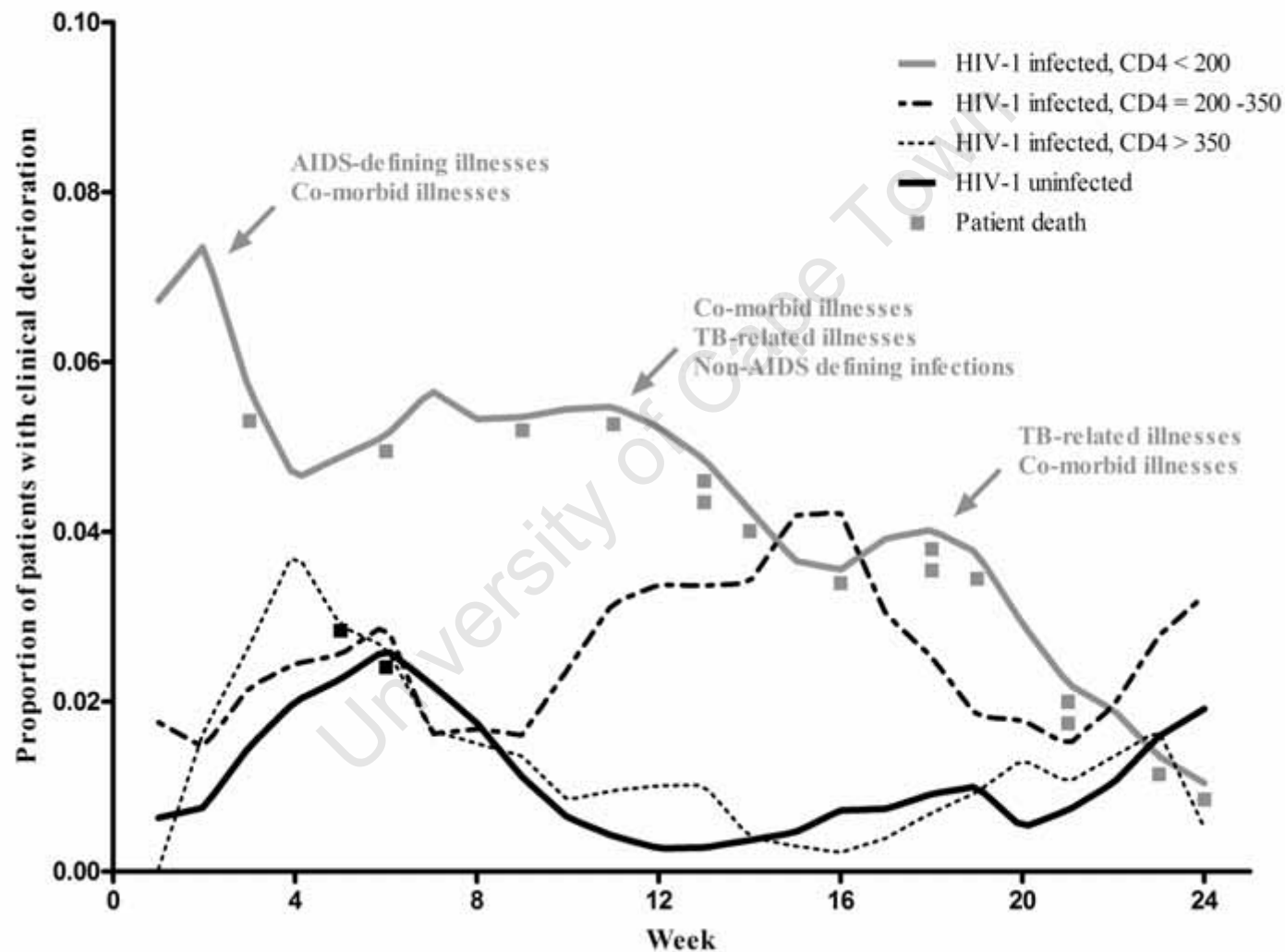
Figure 4.3 is a Lowess plot showing the proportion of patients who experienced clinical deterioration during the 24 weeks of antituberculosis treatment. The initial peak at 6 weeks in HIV-1 uninfected patients corresponds with tuberculosis-related illnesses (mostly paradoxical reactions) and the baseline fluctuations represent co-morbid illnesses. The curve for HIV-1 infected patients with a CD4⁺ count > 350 cells/ μ L is similar to that of HIV-1 uninfected patients, despite an earlier peak for paradoxical reactions. The proportion of HIV-1 infected patients with a CD4⁺ count of 200–350 cells/ μ L who experienced clinical deterioration substantially increased after 10 weeks of follow-up. Furthermore, a substantially higher proportion of HIV-1 infected patients with a CD4⁺ count <200cells/ μ L experienced clinical deterioration compared to other CD4⁺ strata.

Mortality

Fifteen of 17 deaths occurred in HIV-1 infected patients with a CD4+ count < 200cells/ μ L. The median interval from antituberculosis treatment to death was 98 days (IQR= 59 -128). Eight of 17 deaths occurred between 10 and 20 weeks of follow-up. Diagnoses at time of death included: AIDS-defining illnesses (5), poor adherence with antituberculosis treatment (4), paradoxical neurologic TB-IRIS (3), enteric illness (2), MDR-TB (1), pulmonary embolus (1) and tension pneumothorax (1).

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Figure 4.3: Lowess plot showing the proportion of patients who experienced clinical deterioration during 24 weeks of antituberculosis treatment



Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Number in follow-up																								
HIV-1 uninfected	79	76	76	74	74	72	72	71	69	68	66	65	64	63	62	60	59	59	57	57	56	55	54	53
HIV-1 infected, CD4+ > 350	36	35	35	35	33	33	33	31	31	31	31	30	30	30	29	28	28	28	28	26	24	24	24	24
HIV-1 infected, CD4+ = 200-350	37	37	37	37	37	37	37	36	36	36	36	36	36	36	35	34	32	32	32	32	32	32	32	31
HIV-1 infected, CD4+ < 200	132	129	126	126	124	125	125	125	122	116	116	114	112	111	109	107	106	103	100	99	96	94	93	92
Number with deterioration																								
HIV-1 uninfected	1	0	1	2	1	3	2	2	1	0	0	0	0	0	0	1	0	0	2	0	0	0	1	1
HIV-1 infected, CD4+ > 350	0	1	0	3	1	2	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0
HIV-1 infected, CD4+ = 200-350	1	0	1	1	0	3	0	1	0	0	1	2	2	0	1	3	1	1	0	1	0	0	1	1
HIV-1 infected, CD4+ < 200	6	12	10	3	4	3	10	7	6	6	7	6	6	6	4	2	3	4	6	4	1	2	1	1
Percentage with deterioration																								
HIV-1 uninfected	1.3	0.0	1.3	2.7	1.4	4.2	2.8	2.8	1.4	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	3.5	0.0	0.0	0.0	1.9	1.9
HIV-1 infected, CD4+ > 350	0.0	2.9	0.0	8.6	3.0	6.1	0.0	0.0	3.2	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	3.8	0.0	0.0	4.2	0.0
HIV-1 infected, CD4+ = 200-350	2.7	0.0	2.7	2.7	0.0	8.1	0.0	2.8	0.0	0.0	2.8	5.6	5.6	0.0	2.9	8.8	3.1	3.1	0.0	3.1	0.0	0.0	3.1	3.2
HIV-1 infected, CD4+ < 200	4.5	9.3	7.9	2.4	3.2	2.4	8.0	5.6	4.9	5.2	6.0	5.3	5.4	5.4	3.7	1.9	2.8	3.9	6.0	4.0	1.0	2.1	1.1	1.1
Number of deaths																								
HIV-1 uninfected	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV-1 infected, CD4+ > 350	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV-1 infected, CD4+ = 200-350	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV-1 infected, CD4+ < 200	0	0	1	0	0	1	0	0	1	0	1	0	2	1	0	1	0	2	1	0	2	0	1	1

4.3 Discussion

During 24 weeks of follow up, 40% of patients experienced clinical deterioration. In multivariate analyses, significant risk factors for clinical deterioration were HIV-1 infection and a low CD4+ count at tuberculosis diagnosis. The relative risk of clinical deterioration in HIV-1 infected patients did not differ significantly in two of three CD4+ strata (CD4+ count of 200 - 350 cells/ μ L and < 200 cells/ μ L). However, the incidence rate of clinical deterioration was significantly higher in those with a CD4+ count < 200 cells/ μ L. This difference can be attributed to multiple illnesses occurring in profoundly immune suppressed patients.

A distinct pattern of clinical deterioration emerged during the 24 weeks of follow-up (Figure 3). After 10 weeks of follow-up, we observed a rise in the proportion of patients with a CD4+ count of 200 -350 cells/ μ L who experienced clinical deterioration. Few of these patients had initiated ART (according to national protocol [SATB guidelines 2004]). Further studies are needed to determine whether ART initiated soon after tuberculosis diagnosis in this subgroup could reduce the incidence of clinical deterioration. A triple wave of illnesses occurred in profoundly immune-suppressed HIV-1 infected patients (CD4+ count < 200 cells/ μ L). The first wave (0–4 weeks) comprised co-morbid illnesses and AIDS-defining illnesses (data not presented). This highlights the profound immune-suppression at tuberculosis diagnosis and the rapid occurrence of AIDS-defining illnesses soon thereafter. The second wave represented co-morbid illnesses, tuberculosis-related illnesses and non AIDS-defining HIV-1 related infections, while the third wave included tuberculosis-related and co-morbid illnesses. This demonstrates that immune restoration during antituberculosis treatment and ART is not without complications (Meintjes 2008). The substantial occurrence of co-morbid illnesses throughout the 24 weeks of follow-up was unexpected. Hepatic, renal and cardiovascular related morbidity and mortality are well described in HIV-infected patients (El Sadr 2006). Similarly, co-morbid

illnesses causing death in HIV-1 uninfected tuberculosis patients have also been reported (Sterling 2006). However, the magnitude of co-morbid illnesses in HIV-1 infected patients receiving antituberculosis treatment has not previously been reported. The plethora of illnesses and the 15 deaths (related to AIDS-defining illnesses, poor adherence, and neurologic TB-IRIS) suggest that the management of tuberculosis in profoundly immune-suppressed HIV-1 infected patients is complicated.

Due to the observational nature of our study, we were unable to compare the incidence of clinical deterioration *after* ART initiation in two groups of tuberculosis patients: those with a CD4⁺ count of 200-350 cells/ μ L and those with a CD4⁺ count < 200cells/ μ L. Only patients in the latter group were eligible for ART according to South African guidelines (SATB guidelines 2004). Further interventional research is needed to determine whether initiation of ART after tuberculosis diagnosis reduces the incidence of clinical deterioration in patients with a CD4⁺ count of 200 -350 cells/ μ L.

Fewer deaths than anticipated occurred during the first 8 weeks [5]; many deaths (8 of 17) occurred from weeks 10 to 20 of follow-up. It is possible that patients with severe tuberculosis may have died in the referral hospital, precluding enrolment in our study. Patients requiring hospital admission for fatal AIDS-defining illnesses at tuberculosis diagnosis would similarly have not been enrolled. Ninety-two (32%) of 292 patients were diagnosed with tuberculosis at hospital, suggesting that tuberculosis disease at tuberculosis diagnosis was severe.

Bacterial pneumonia is an important cause of hospitalisation and death in HIV-1 infected patients with (Corbett 2002), or without (Martinson 2007) a diagnosis of tuberculosis. In our clinic-based cohort, only one patient with pneumonia cultured a bacterial organism (*H.*

influenzae). We attribute this finding to the combined antibacterial properties of rifampin and trimethoprim-sulfamethoxazole (TMP-SMX) (Dirienzo 2002). Most HIV-1 infected patients (86%) in our study received TMP-SMX chemoprophylaxis (160/ 800mg daily).

We diagnosed symptomatic drug-induced hepatitis in one patient. Drug-induced hepatitis, defined as a 5-fold rise in liver enzymes (AST or ALT), is reported in 2-28% of patients receiving antituberculosis treatment (Tostmann 2008) and 6% of HIV-1 infected patients receiving both antituberculosis and antiretroviral treatment (Dean 2002). In our study, liver function tests were not performed in asymptomatic patients receiving antituberculosis treatment or efavirenz-based ART, according to routine practice. Liver function tests were performed when patients experienced clinical deterioration.

During the course of our study, DST was performed at tuberculosis diagnosis, at 8 and 20 weeks of follow-up and at clinical deterioration. This differs from routine practice in South Africa where only certain patients (such as those receiving retreatment for tuberculosis and those who do not respond to antituberculosis treatment – SATB guidelines 2004) receive DST due to resource constraints. The ability to perform DST in all patients in our study, regardless of previous tuberculosis, likely expedited diagnosis and appropriate management of MDR-TB, especially where the differential diagnoses included MDR-TB and TB-IRIS.

Our study has some limitations. Our definition of clinical deterioration (symptomatic worsening or failure to stabilise within 24 weeks after initiation of antituberculosis treatment) did not include episodes of clinical deterioration that occurred after 24 weeks of follow-up. Twenty-four weeks of follow-up is a short period of observation. It is possible that the causes for deterioration could differ after 24 weeks of follow-up. The relatively low number of

patients diagnosed with MDR-TB during follow-up (n=5) may underestimate the incidence of MDR-TB presenting after 24 weeks. It is noteworthy that 6 patients were diagnosed with MDR-TB during the 24 weeks of follow-up without fulfilling our definition of clinical deterioration. We have previously described the phenomenon of initial clinical improvement with rifampin-resistant *M. tuberculosis* despite receiving standard antituberculosis treatment (Meintjes 2009). At diagnosis of MDR-TB in these 6 patients, we appropriately intensified their antituberculosis treatment and no subsequent clinical deterioration occurred. We also excluded isolated radiological worsening from our case definition of clinical deterioration. Thus, our finding of clinical deterioration in 40% of patients may be an underestimate. A substantial proportion of patients were lost to follow-up. The median CD4+ count among HIV-1 infected patients who were lost to follow-up was 150 cells/ μ L (IQR= 67-338). Chapter 5 describes risk factors for loss to follow-up. Also, there were some patients in whom the cause of deterioration could not be identified. Our study did not evaluate the proportion of patients with known HIV-1 status prior to tuberculosis diagnosis. Patients who we initially assessed at the tuberculosis clinic, who subsequently presented to the hospital, and then deteriorated and died during their first hospital admission, were included in the analysis. It is possible that some patients within the tuberculosis clinic's catchment area were not included in the study. These patients might have died prior to assessment at the tuberculosis clinic. Lastly, this study was conducted at a tuberculosis clinic and a referral hospital that serve a large population with a high incidence of HIV-1-associated tuberculosis; thus, our findings may not be generalised to other settings.

Chapter 5

The Challenge of Loss to Follow-up during Antituberculosis Treatment in HIV-1 infected patients in South Africa

University of Cape Town

Contributions: The study was conceived and designed by the candidate, Robert J. Wilkinson and Graeme Meintjes. Data was collected by the candidate, Suzaan Marais and Graeme Meintjes. Data was interpreted by the candidate, Suzaan Marais, Robert J. Wilkinson, Feriyl Bhaijee and Graeme Meintjes. The candidate prepared the initial draft, which was critically revised by Suzaan Marais, Robert J. Wilkinson, Feriyl Bhaijee and Graeme Meintjes.

Publications: The manuscript arising from this chapter is yet to be submitted for publication, as of 5 May 2010.

In chapter 4, we found that clinical deterioration during antituberculosis treatment was significantly associated with profound immune-suppression in HIV-1 infected patients. Here, at 24 weeks follow-up, we determined the clinical outcomes of the same cohort of adult HIV-1 infected patients who received antituberculosis treatment. We also determined factors associated with death, loss to follow-up and not receiving ART.

5.1 Methods

We have previously described our cohort of patients, the study site, the study setting and the study definitions in chapter 4. In this analysis, we determined the clinical outcomes of 209 HIV-1 infected patients. The Research Ethics Committee of the University of Cape Town approved this study (REC 178/2008).

Assessment of outcomes. We followed 209 HIV-1 infected patients for 24 weeks after initiation of antituberculosis treatment. The primary objectives were:

1. The clinical outcome at 24 weeks, and
2. The proportion of patients not receiving ART during follow-up.

We classified clinical outcomes as: i) alive and in follow-up, ii) dead, and iii) lost to follow-up. We defined a patient as ‘lost to follow-up’ if we were unable to trace a patient 24 weeks after initiation of antituberculosis treatment. We used clinic and hospital medical notes, as well as the Provincial Government of the Western Cape’s electronic tuberculosis register to trace patients and record clinical outcomes. We performed statistical analyses using Stata 10.0 (Texas, USA). Wilcoxon rank-sum and Kruskal-Wallis tests were used for group comparisons, and Fisher’s exact tests to compare proportions.

The secondary objective was to determine risk factors related to death, loss to follow-up and not receiving ART. We used multivariate logistic regression models to determine significant ($p < 0.05$) risk factors. Variables considered in the analysis were: demographic information (age, sex), tuberculosis information (previous tuberculosis, microbiologic confirmation with drug susceptibility testing, diagnosis at a tuberculosis clinic, presence of only pulmonary tuberculosis, weight at tuberculosis diagnosis), HIV-1 information (CD4+ count at tuberculosis diagnosis, TMP-SMX chemoprophylaxis, ART), and operational information (assessed by a tuberculosis doctor, referred to an ART clinic, experienced an episode of clinical deterioration, admitted to hospital). Variables with the outcome of interest were entered into logistic regression models to assess independent effects of covariates. Variables having a significant association with the outcome of interest were removed from the model to ensure no other variables were significant.

5.2 Results

Descriptive analyses of outcomes

ART initiation. Of 209 patients, 34 (16%) patients received ART prior to tuberculosis diagnosis, 75 (36%) initiated ART during the 24 weeks of follow-up and 100 (48%) did not receive ART during follow-up (figure 5.1). In patients who received ART prior to tuberculosis diagnosis, the interval from ART initiation to antituberculosis treatment was 359 days (median, IQR=164-872) and the CD4+ count at tuberculosis diagnosis was 242 cells/ μ L (median, IQR: 151-395). In patients who initiated ART during the 24 weeks of follow-up, the interval from antituberculosis treatment to ART initiation was 60 days (median, IQR=37-90) and the CD4+ count at tuberculosis diagnosis was 66 cells/ μ L (median, IQR: 30-110).

Eligibility for ART. Of 175 patients not receiving ART at tuberculosis diagnosis, 121 (69%) were eligible for ART according to South African ART guidelines. Of 121 eligible patients, 71 (59%) initiated ART during 24 weeks of follow-up, while 50 (31%) did not.

Loss to follow-up. Forty-six (22%) patients were lost to follow-up. The interval from antituberculosis treatment to loss to follow-up was 80 days (median, IQR=37-126). The median CD4+ count among HIV-1 infected patients who were lost to follow-up was 150 cells/ μ L (IQR= 67-338).

Death. Sixteen (8%) patients died during follow-up. Diagnoses at death were AIDS-defining illnesses (5), poor compliance with antituberculosis treatment (3), paradoxical neurological TB-IRIS (3), enteric illness (2), MDR-TB (1), pulmonary embolus (1) and tension pneumothorax (1). The interval from commencing antituberculosis treatment to death was 98 days (median, IQR=59-128).

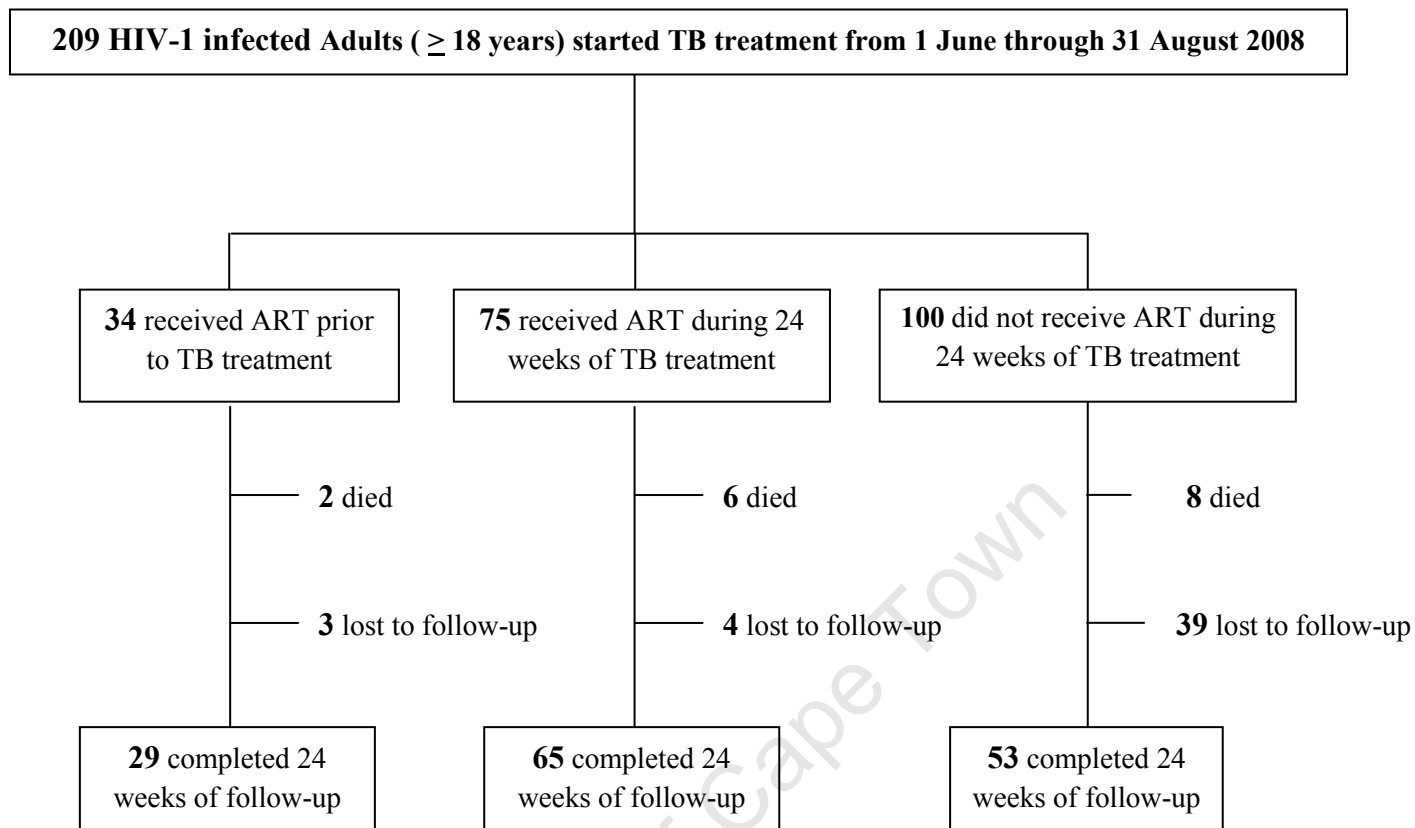


Figure 5.1: Flow diagram showing the 24 week clinical outcomes of 209 HIV-1 infected Patients with Tuberculosis

Univariate and logistic regression analyses of outcomes

Death. In univariate analysis (table 5.1), three factors were significantly associated with death: a lower CD4+ count at tuberculosis diagnosis, occurrence of clinical deterioration, and admission to hospital during follow-up. In multivariate analysis (table 5.3), only a lower CD4+ count at tuberculosis diagnosis remained significant (RR = 4.0, 95% CI=1.06–14.67).

Loss to follow-up. In univariate analysis (table 5.1), five factors were significantly associated with loss to follow-up: younger age, lower weight at tuberculosis diagnosis, failure to be referred to the HIV clinic, ART not received, and absence of clinical deterioration. In multivariate analysis (table 5.3), which did not include the collinear variable ‘failure to be referred to the HIV clinic’, only ‘ART not received’ remained significant (RR = 7.0, 95% CI=2.60–19.01).

ART not received during the 24 weeks of follow-up. In univariate analysis, 9 factors were significantly associated with the failure to initiate ART during 24 weeks of follow-up: a higher CD4+ count, microbiological confirmation of tuberculosis (with DST) at tuberculosis diagnosis, diagnosis of tuberculosis at the tuberculosis clinic, absence of extra-pulmonary tuberculosis, not receiving TMP-SMX chemoprophylaxis, failure to be assessed by a tuberculosis doctor, failure to be referred to the HIV-1 clinic, absence of clinical deterioration and loss to follow-up. In multivariate analysis (table 5.3), only a CD4+ count greater than 200 cells/ μ L remained significant (RR = 5.5 [95% CI=1.77–16.91]). Figure 5.2 depicts Kaplan-Meier plots, which show i) the proportion of patients lost to follow-up, according to whether they received ART or not, and ii) the proportion of patients who died, according to CD4+ stratum.

Table 5.1: Univariate risk factor analysis for 24 week outcomes in 209 HIV-1 infected patients who received antituberculosis treatment									
	Alive	Lost to follow-up⁴				Died⁵			
	(n=147)	(n=46)	p-value	RR	95% CI	(n=16)	p-value	RR	95% CI
Female, n (%)	80 (54)	26 (57)	0.866	1.1	0.64 – 1.78	6 (38)	0.292	0.5	0.20 – 1.41
Age in years, median (IQR)	36 (30 - 43)	32 (30 - 38)	0.041			33 (29 - 36)	0.158		
Previous TB, n (%)	51 (35)	15 (33)	0.860	0.9	0.54 – 1.60	5 (31)	1.000	0.9	0.32 – 2.38
CD4 count in cells/μL, median (IQR)	144 (66 - 272)	150 (67 - 347)	0.362			34 (26 - 89)	0.003		
Weight at TB diagnosis in kg, median (IQR)	55 (49 - 66)	52 (48 - 59)	0.035			52 (45 - 56)	0.063		
TB diagnosed at hospital, n (%)	55 (37)	16 (35)	0.861	0.9	0.54 – 1.56	8 (50)	0.419	1.6	0.63 – 4.01
Extra-pulmonary TB, n (%)	62 (42)	13 (28)	0.119	0.6	0.35 – 1.10	7 (44)	1.000	1.1	0.41 – 2.71
Microbiologic confirmation at TB diagnosis ¹ , n (%)	84 (57)	28 (61)	0.733	1.1	0.67 – 1.89	11 (69)	0.433	1.6	0.57 – 4.32
Microbiologic confirmation with drug susceptibilities known at TB diagnosis ¹ , n (%)	40 (27)	21 (46)	0.028	1.8	1.11 – 2.98	6 (38)	0.391	1.5	0.59 – 3.96
TMP-SMX ² chemoprophylaxis, n (%)	128 (87)	40 (87)	1.000	1.0	0.47 – 2.10	13 (81)	0.456	0.7	0.21 – 2.18
Referred to HIV Clinic	114 (78)	23 (50)	< 0.001	0.4	0.25 – 0.67	9 (56)	0.071	0.4	0.17 – 1.05
ART ³ during TB chemotherapy, n (%)	93 (63)	8 (17)	0.018	0.2	0.09 – 0.39	8 (50)	0.416	0.6	0.24 – 1.55
Experienced clinical deterioration, n (%)	74 (50)	13 (28)	0.011	0.5	0.27 – 0.85	16 (100)	< 0.001	> 2	
Hospital admission during 24 weeks of TB chemotherapy, n (%)	49 (33)	15 (33)	1.000	1.0	0.57 – 1.67	16 (100)	< 0.001	> 2	
Microbiologic confirmation at 24 weeks, n (%)	104 (71)	35 (76)	0.574	1.2	0.68 – 2.25	14 (88)	0.239	2.7	0.63 – 11.28
Microbiologic confirmation with drug susceptibilities known at 24 weeks, n (%)	77 (52)	29 (63)	0.237	1.4	0.83 – 2.37	11 (69)	0.292	1.9	0.68 – 5.15

¹ No TB specimens sent in 12 HIV-1 infected patients and 3 HIV-1 uninfected patients at TB diagnosis;
² TMP-SMX = trimethoprim sulfamethoxazole 160/800mg daily;
³ Antiretroviral treatment regimens were as follows: D4T/3TC/EFV (89 patients), D4T/3TC/NVP (3 patients), AZT/3TC/EFV (13 patients), AZT/3TC/NVP (2 patients), TDF/3TC/EFV (2 patients); D4T= stavudine 30mg twice daily, 3TC = lamivudine 150mg twice daily, EFV = efavirenz 600mg nocte, AZT = zidovudine 300mg twice daily, NVP = nevirapine 200mg twice daily, TDF = tenofovir 300mg daily; TB = Tuberculosis, RR = relative risk, 95% CI = 95% confidence interval
⁴ Risk factor analysis compared patients who were lost to follow-up against patients who were alive at 24 weeks ⁵ Risk factor analysis compared patients who died against patients who were alive at 24 weeks

Table 5.3: Logistic regression analysis in 209 HIV-1 infected tuberculosis patients showing significant risk factors in multivariate analysis for loss to follow-up, death and not receiving ART during 24 weeks of follow-up

	p-value	RR	95% CI
Loss to follow-up			
Antiretroviral treatment not received during 24 weeks of follow-up ¹	< 0.001	7.0	2.60 – 19.01
Death			
Lower CD4+ stratum (relative to stratum 1) ²	0.040	4.0	1.06 – 14.67
ART not received during follow-up			
CD4+ count > 200 cells/ μ L at this tuberculosis episode	0.003	5.5	1.77 – 16.91
¹ Antiretroviral treatment regimens were as follows: D4T/3TC/EFV (89 patients), D4T/3TC/NVP (3 patients), AZT/3TC/EFV (13 patients), AZT/3TC/NVP (2 patients), TDF/3TC/EFV (2 patients); D4T= stavudine 30mg twice daily, 3TC = lamivudine 150mg twice daily, EFV = efavirenz 600mg nocte, AZT = zidovudine 300mg twice daily, NVP = nevirapine 200mg twice daily, TDF = tenofovir 300mg daily; ² CD4+ strata used (CD4+ counts not performed in 3 of 209 HIV-1 infected patients): Stratum 1 = CD4+ > 350 cells/ μ L (36 patients) Stratum 2 = CD4+ from 200 – 350 cells/ μ L (37 patients) Stratum 3 = CD4+ < 200 cells/ μ L (133 patients) RR = relative risk, 95% CI = 95% confidence interval			

Figure 5.2a: Kaplan Meier plot showing the proportion of HIV-1 infected TB patients lost to follow-up, according to whether antiretroviral treatment was received or not

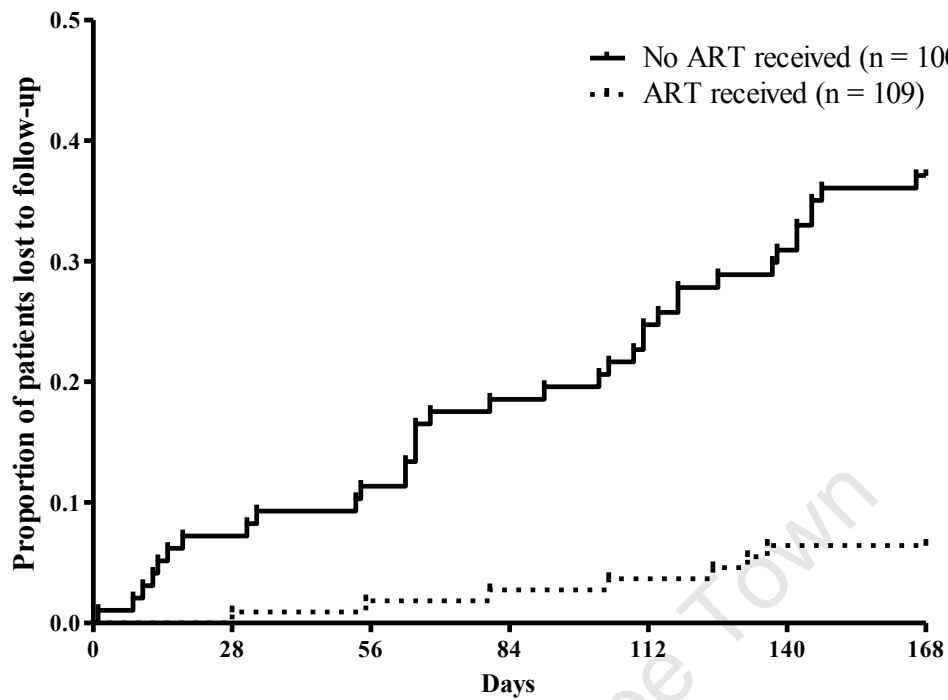
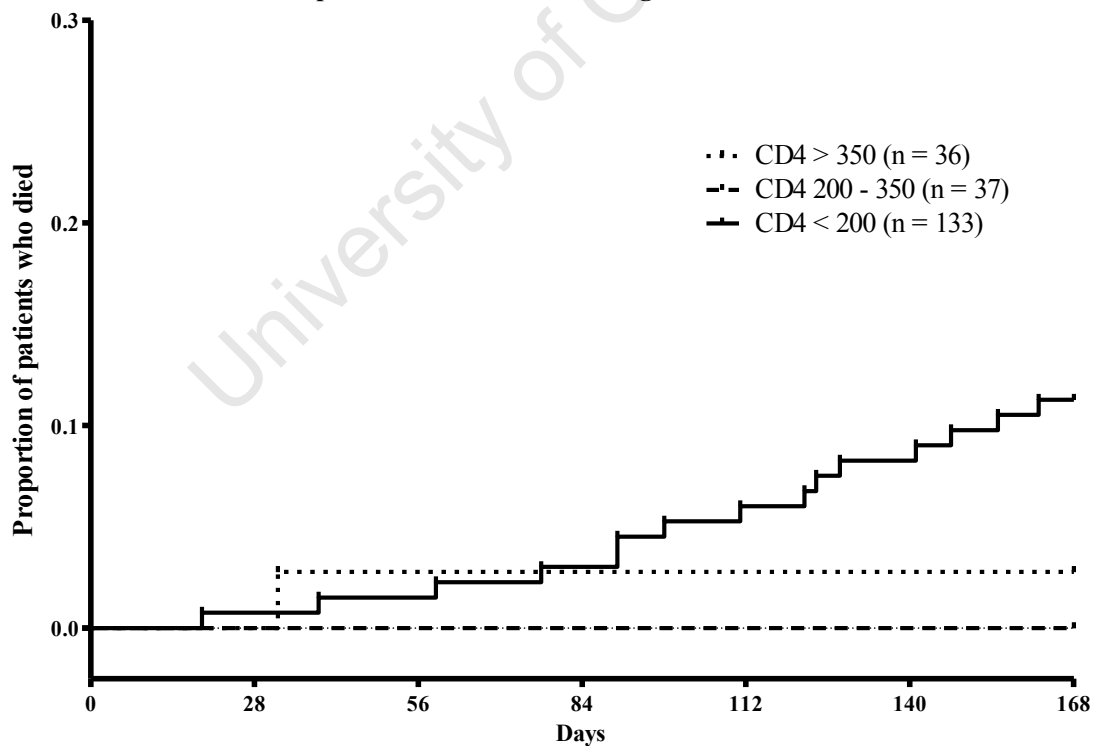


Figure 5.2b: Kaplan Meier plot showing the proportion of HIV-1 infected TB patients who died, according to CD4 stratum*



5.3 Discussion

We conducted a prospective, clinic-based study in an urban setting in sub-Saharan Africa, in which we followed 209 HIV-1 infected patients for 24 weeks after initiation of antituberculosis treatment. We found that 70% of patients completed follow-up, 22% of patients were lost to follow-up and 8% of patients died. We found a significant association between not receiving ART and being lost to follow-up. We also showed that patients were less likely to initiate ART if they were not profoundly immune-suppressed (CD4+ count greater than 200 cells/ μ L at tuberculosis diagnosis), in keeping with national guidelines (SATB guidelines 2004). Lastly, we confirmed that profound immune-suppression increases the risk of death.

Our study adds to previous observational data detailing the benefits of ART during antituberculosis treatment. Our study's strengths include its prospective design, its nesting within a drug susceptibility testing survey and the use of multivariate analysis, which incorporated a number of demographic, HIV-1, tuberculosis and operational factors. Although not novel, our study resonates with previous studies, which describe the effect of profound immune-suppression on clinical outcome.

Eight percent of our HIV-1 infected patients with tuberculosis died during the 24 weeks of follow-up. In sub-Saharan Africa, up to 30% of HIV-1 infected patients die during antituberculosis treatment (Harries 2001). In South-East Asia, 7 – 30% of HIV-1 infected patients die during antituberculosis treatment (Akksilp 2007, Cain 2007, Quy 2006, Thuy 2007). We attribute our low mortality to early ART initiation. Amongst the 75 patients who initiated ART during antituberculosis treatment, the CD4+ count at tuberculosis diagnosis was very low (median of 66 cells/ μ L, IQR: 30-110), but the interval from antituberculosis treatment to ART initiation was relatively short (median of 60 days, IQR=37-90). While the

benefits of ART in restoring pathogen-specific immunity and preventing further immune suppression are well described (Akksilp 2007, Karim 2009), the optimal timing of ART during antituberculosis treatment has yet to be determined. It is concerning that in our study, 31% of patients who were eligible for ART at tuberculosis diagnosis did not initiate ART within 24 weeks. Reasons for not initiating ART in eligible patients require investigation in future studies. Alternative explanations for our low mortality rate do exist: Our study was clinic-based so patients with fatal tuberculosis may not have been enrolled if they required immediate referral to hospital at initial presentation; also, a substantial proportion of patients (22%) was lost to follow-up and may have died. We consider the latter less likely as written and electronic charts – recording deaths from the referral hospitals, tuberculosis clinic and ART clinic – were assessed on 3 separate occasions following conclusion of the study.

In multivariate analysis, we found death to be associated with profound immune-suppression i.e. a CD4⁺ count of less than 200 cells/ μ L. We found that deaths were predominantly due to AIDS-defining illnesses, poor compliance with antituberculosis treatment and neurologic TB-IRIS. Neurologic TB-IRIS is regarded as one of the more severe manifestations of paradoxical TB-IRIS (Pepper 2009). Other studies have shown that ART reduces deaths due to tuberculosis and HIV-1 related illnesses (Manosuthi 2006).

The high proportion of patients lost to follow-up (22%) was unexpected and concerning. International literature reports that 7-9% of HIV-1 infected patients are lost to follow-up during antituberculosis treatment (Varma 2009, Makombe 2007). This likely represents publication bias. Risk factors for loss to follow-up are difficult to ascertain. In multivariate analysis, we found that loss to follow-up was positively associated with not receiving ART. It is not known whether loss to follow-up was responsible for patients not receiving ART, or whether the

failure to start ART in these patients resulted in loss to follow-up. The latter is certainly plausible: the occurrence of profound immune-suppression with life-threatening illnesses necessitates ART initiation. This likely improves follow-up as ART programmes offer a safety net of counselling and medical support. Studies have shown the benefit of dual therapy: ART with antituberculosis treatment is associated with increased retention during follow-up when compared to ART alone (Akksilp 2007). In our study, the positive association of receiving ART and retaining patients merits further research. If confirmed in other similar settings, the reasons for this association need to be determined.

Our study shows that the only significant barrier to initiating ART is the current national protocol, which offers ART at CD4+ counts of less than 200 cells/ μ L (SA TB guidelines 2004). This barrier exists despite knowledge that ART improves the survival of HIV-1 infected patients with CD4+ counts up to 350 cells/ μ L. In December 2009, the South African government announced its intention to provide ART to all pregnant mothers and those with tuberculosis provided the CD4+ count is less than 350 cells/ μ L. This intention is yet to be implemented.

We acknowledge certain limitation in this study. Referral bias is inevitable; in a clinic-based study, patients with fatal tuberculosis who required hospital admission may not have been enrolled. In addition, we were unable to assess the reasons for loss to follow-up. In our setting, the following obstacles preclude investigation of reasons for loss to follow-up: 1) Most patients reside in informal housing ('shacks') and the addresses of informal houses are vulnerable to change with inclement weather and the building of formal settlements. 2) Cellular/ mobile telephones are the preferred method of contacting patients in informal housing, but these telephones are subject to theft and loss. 3) Anecdotal reports from health-care nurses indicate a

dynamic flux of patients between Cape Town and the Eastern Cape Province (located 1000km eastward of Cape Town). Patients may have returned to the Eastern Cape Province and received health care or died there. Future studies are needed to determine the reasons for loss to follow-up.

In conclusion, we demonstrate the importance of ART initiation in HIV-1 infected patients with tuberculosis. We also show that loss to follow-up is a significant problem and that ART is underutilised. ART and tuberculosis programmes need to be strengthened in South Africa. In chapter 7, I synthesise the findings of chapters 3, 4 and 5 and offer potential solutions.

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Chapter 6

Neurologic Manifestations of Paradoxical Tuberculosis- Associated Immune Reconstitution Inflammatory Syndrome: A Case Series

University of Cape Town

Contributions: The study was conceived by the candidate, Robert J. Wilkinson and Graeme Meintjes. The study was designed by the candidate, Suzaan Marais, Robert J. Wilkinson and Graeme Meintjes. The data was obtained by the candidate, Suzaan Marais, Kevin Rebe, Molebogeng Rangaka, Tolu Oni and Graeme Meintjes. The data was interpreted by the candidate, Suzaan Marais, Gary Maartens, Kevin Rebe, Chelsea Morroni, Molebogeng Rangaka, Tolu Oni, Robert J. Wilkinson and Graeme Meintjes. The candidate prepared the first draft, which was critically revised by Suzaan Marais, Gary Maartens, Kevin Rebe, Chelsea Morroni, Molebogeng Rangaka, Tolu Oni, Robert J. Wilkinson and Graeme Meintjes.

Publications: **Pepper DJ**, Marais S, Maartens G, Morroni C, Rebe K, Rangaka MX, Oni T, Wilkinson RJ, Meintjes G. Neurological Manifestations of Paradoxical Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome: A Case Series. *Clinical Infectious Diseases*. **2009**; 48 (11):e96-107

In chapters 3 and 4, we found that tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) occurred in 18 – 22% of HIV-1 infected patients who experienced clinical deterioration during antituberculosis treatment. Our frequent interaction with TB-IRIS, its manifestations and its clinical course suggests that TB-IRIS is not the benign condition reported in the literature. In our prospective cohort study, neurologic TB-IRIS was responsible for three of seventeen deaths. In this chapter, we share our experiences of this clinical entity.

6.1 Introduction

Paradoxical and unmasking TB-IRIS (Meintjes 2008) are emerging complications of ART in countries with high rates of tuberculosis, especially in Africa (Meintjes 2009). The temporal sequence of events distinguishes paradoxical TB-IRIS from unmasking TB-IRIS:

antituberculosis treatment precedes ART in paradoxical TB-IRIS, whereas ART precedes tuberculosis diagnosis in unmasking TB-IRIS (Meintjes 2008). Currently, there is no confirmatory diagnostic test for paradoxical TB-IRIS. Differential diagnoses include: failure of antituberculosis treatment due to antimicrobial resistance or suboptimal antituberculosis drug concentrations, drug reactions, and alternative opportunistic conditions (McIlleron 2006, Pepper 2007). Published case definitions require the exclusion of these differential diagnoses, if possible, before diagnosis of paradoxical TB-IRIS (Meintjes 2008, Colebunders 2006, Shelburne 2006). Severe and life-threatening manifestations of paradoxical TB-IRIS include respiratory failure (Fishman 2000, Buckingham 2004) and neurologic involvement (Burman 2007, Lee 2007, Dautremer 2007, Sumner 2003, and Vidal 2003). Several case reports of paradoxical neurologic TB-IRIS have been published (Burman 2007, Lee 2007, Dautremer 2007, Sumner 2003, and Vidal 2003); this is the first case series.

In paradoxical neurologic TB-IRIS, inflammation in the central nervous system (CNS) may result in death or permanent neurologic disability. Adjunctive corticosteroid therapy is often used to treat neurologic TB-IRIS, despite a dearth of evidence indicating benefit. Determining the cause of neurologic deterioration in patients with tuberculosis who are receiving ART is important; inappropriate adjunctive corticosteroid therapy for profoundly immune-suppressed patients with sub-optimally treated tuberculosis or other untreated opportunistic infections may be fatal. However, if treatment with corticosteroids is effective, then failure to administer them may have severe consequences. Unfortunately, access to expensive tests, such as neuroimaging and drug susceptibility testing, is poor in resource-limited settings where most of the burden of disease exists.

In this study, we evaluated patients with suspected TB-IRIS who were referred to our hospital for investigation of neurologic deterioration. We used a published consensus clinical case definition of paradoxical TB-IRIS for resource-limited settings (Meintjes 2008) to identify paradoxical neurologic TB-IRIS. Here, we describe the clinical presentation, management, and outcomes of paradoxical neurologic TB-IRIS. We also discuss the challenges of diagnosis and management in resource-limited settings.

University of Cape Town

6.2 Methods

Study site and setting. We conducted a prospective study at GF Jooste Hospital (Cape Town, South Africa) from 1 June 2005 through 31 October 2007. The study site, the study setting, and the national protocols for treating HIV and tuberculosis were described in chapters 2 and 3. The Research Ethics Committee of the University of Cape Town approved this study (REC 337/2004).

Definitions. Patients with suspected TB-IRIS were defined as patients with HIV-1 infection who received antituberculosis treatment, then commenced ART, and subsequently deteriorated within 3 months, with symptoms or signs of tuberculosis disease. Patients with suspected neurologic TB-IRIS were defined as having at least one new/ recurrent neurologic symptom and/or sign, which included: headache, focal neurologic deficit, nuchal rigidity, confusion, seizures, cerebellar signs, cognitive impairment, and/or psychiatric manifestations.

We adapted the consensus clinical case definition of paradoxical TB-IRIS for resource-limited settings (Meintjes 2008). We expanded the major neurologic criterion of this case definition (i.e., new or worsening CNS tuberculosis, including meningitis or focal neurologic deficit [e.g., caused by tuberculoma]) to include the following 3 neurologic disease categories:

- (1) new or worsening tuberculous meningitis;
- (2) new or worsening intracerebral space-occupying lesion (probable tuberculoma); and
- (3) new or worsening radiculomyelopathy.

The consensus clinical case definition requires exclusion of alternative explanations for clinical deterioration, if possible. In a number of cases, we were unable to definitively exclude

alternative explanations for clinical deterioration, but our clinical diagnosis was likely paradoxical neurologic TB-IRIS. We therefore included patients:

- (1) who died despite treatment interventions,
- (2) who, in addition to TB-IRIS treatment, received therapeutic trimethoprim-sulfamethoxazole (320 mg/1600 mg twice daily) to treat possible cerebral toxoplasmosis (the principal differential diagnosis in cases of tuberculoma IRIS), and/or
- (3) in whom antituberculosis treatment was intensified from 2 drugs (isoniazid and rifampin) to 4 drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) or from 4 drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) to 7 drugs (isoniazid, rifampin, pyrazinamide, and ethambutol plus amikacin, ofloxacin, and ethionamide) (in the latter case, to cover for the possibility of drug-resistant tuberculosis).

We regarded all cases of paradoxical neurologic TB-IRIS as ‘probable’, because a confirmatory test for TB-IRIS is not yet available.

We defined the following:

1. Microbiologically confirmed tuberculosis as *Mycobacterium tuberculosis* (*M.tb*) cultured or acid-fast bacilli seen in a sputum, nodal aspirate, or CNS specimen. The CNS specimens included cerebrospinal fluid and brain or paraspinal biopsy specimens.
2. Microbiologically unconfirmed tuberculosis according to World Health Organization case definitions for smear-negative and extra pulmonary tuberculosis (WHO 2007).
3. Multidrug-resistant (MDR) tuberculosis as *M.tb* with resistance to isoniazid and rifampin.
4. Disseminated tuberculosis as tuberculosis disease at ≥ 2 non-contiguous sites or a miliary pattern visible on chest radiograph.

5. HIV-1 encephalopathy as chronic cognitive impairment (with or without cerebral atrophy on computed tomography), with exclusion of opportunistic infections as the cause for neurologic deterioration. Delirium was considered if patients presented with a systemic illness and acute cognitive deterioration. We diagnosed delirium when we observed a return to baseline cognitive function on resolution of systemic illness.
6. Clinical improvement as symptom improvement (e.g., improvement of headache) or improvement assessed by neurologic examination (e.g., focal weakness or Glasgow Coma Scale score improvement from admission). Patients assessed as having initial clinical improvement did not need to have complete resolution of symptoms and/or signs.
7. A residual deficit as persistence of any neurologic symptom or sign.
8. A complete physical and mental recovery as a return to baseline functioning, as assessed by the physician who discharged the patient from the hospital, and lastly
9. A patient as 'lost to follow-up' if we were unable to trace a patient 6 months after initial assessment for neurologic deterioration. We used hospital records, as well as the National Health Laboratories Service and the Provincial Government of the Western Cape's electronic databases, to trace patients.

Assessment of patients with suspected TB-IRIS and data collection. We assessed patients with suspected TB-IRIS from June 2005 through October 2007, during recruitment to a randomized controlled trial of prednisone versus placebo for mild and moderate paradoxical TB-IRIS. Before the trial, clinical case definitions for TB-IRIS were prepared, circulated, and discussed with participating primary care physicians. Patients with suspected TB-IRIS who were referred to GF Jooste Hospital were prospectively assessed (regardless of severity of illness, eventual diagnosis, or inpatient or outpatient management) to exclude differential diagnoses. For

example, we excluded bacterial and cryptococcal meningitis in patients with features that were suggestive of meningitis. We also requested mycobacterial culture and drug susceptibility testing on specimens, such as sputum, cerebrospinal fluid, and nodal aspirates, to exclude drug-resistant *M. tuberculosis*. Patients with severe paradoxical TB-IRIS manifestations, such as respiratory failure, altered level of consciousness, or new focal neurologic signs, were excluded from the randomized controlled trial and often received prednisone. For patients with suspected neurologic TB-IRIS, we collected the following data: tuberculosis diagnosis, ART, CD4+ cell count before and after initiation of ART, details of neurologic deterioration, eventual diagnosis, clinical management, and outcome at 6 months after presentation.

6.3 Results

During the 29-month study period, we assessed 279 patients with suspected TB-IRIS (figure 6.1). Two hundred twenty-five (81%) of 279 patients with suspected TB-IRIS had suspected non-neurologic TB-IRIS, and 54 (19%) had suspected neurologic TB-IRIS on the basis of ≥ 1 neurologic symptom and/or sign. TB-IRIS was diagnosed more often among patients with suspected non-neurologic TB-IRIS than it was among those with suspected neurologic TB-IRIS (166 [74%] of 225 vs. 23 [43%] of 54; $P < 0.001$, by X^2 test). In the 54 patients with suspected neurologic TB-IRIS, we diagnosed paradoxical neurologic TB-IRIS in 23 patients and other illnesses in 31 patients. Therefore, of 190 patients with a diagnosis of paradoxical TB-IRIS, 23 (12%; 95% confidence interval, 7%–17%) had neurologic TB-IRIS: 8 had meningitis, 7 had tuberculoma, 5 had both tuberculoma and meningitis, and 3 had radiculomyelopathy. Among the 31 patients with illnesses other than paradoxical neurologic TB-IRIS, the most frequent diagnoses were HIV-1 encephalopathy with delirium (7 patients), cryptococcal meningitis (5 patients), and MDR tuberculosis (4 patients).

Table 6.1 gives the baseline characteristics and neurologic symptoms and signs for patients with a diagnosis of paradoxical neurologic TB-IRIS. The median CD4+ cell count was 61 cells/ μL (IQR: 29–97 cells/ μL) before ART and 293 cells/ μL (IQR, 128–482 cells/ μL) a median of 183 days (IQR, 161–223 days) after ART initiation. The median duration from antituberculosis treatment to initiation of ART was 65 days (IQR, 41–87 days), and median duration from ART initiation to new or recurrent neurologic symptoms was 14 days (IQR, 5–29 days). The most frequent neurologic symptoms and signs were headache (16 patients; 70%), nuchal rigidity (9 patients; 39%), and seizure(s) (7 patients; 30%). The median duration from onset of neurologic symptoms to initial hospital assessment was 6 days (IQR, 3–14 days).

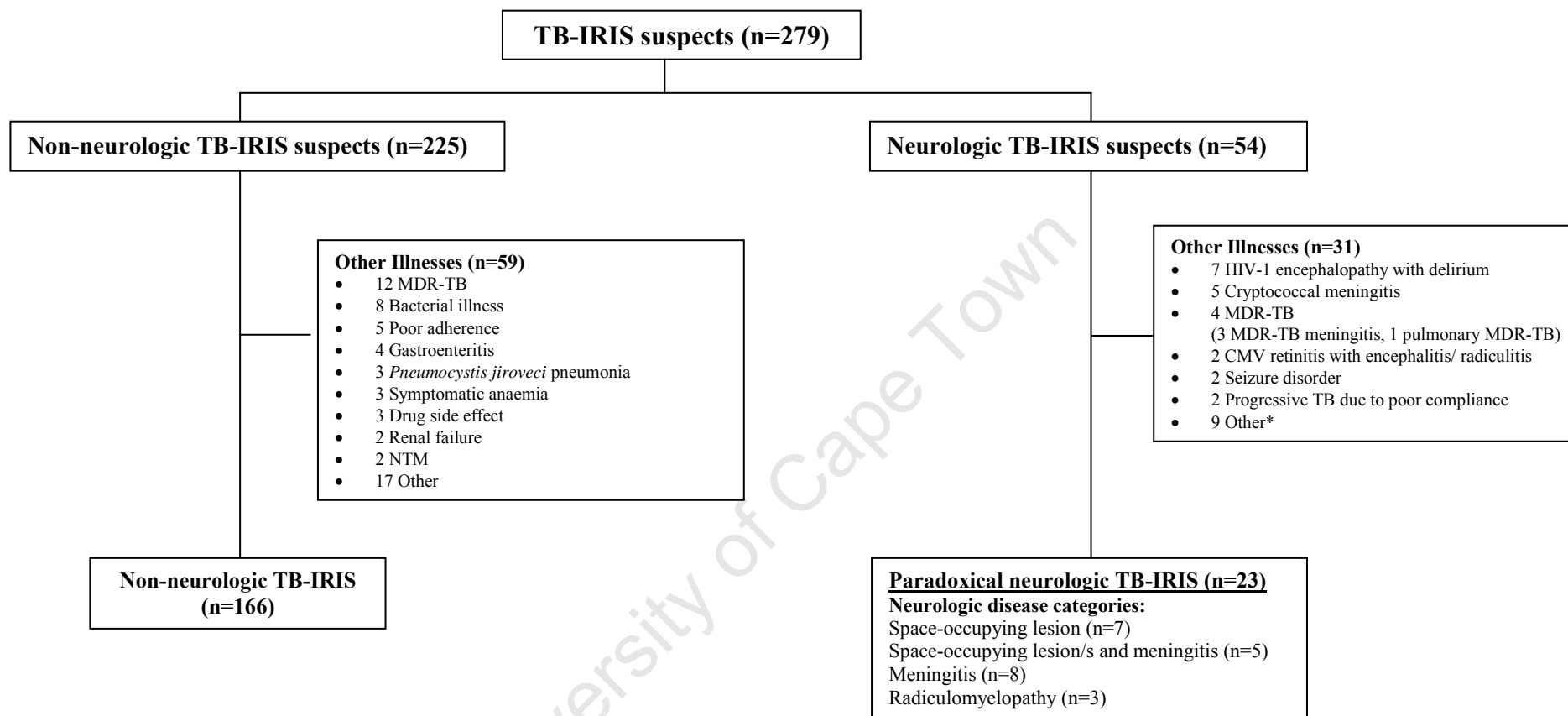


Figure 6.1: Diagnoses of 279 TB-IRIS suspects (1 June 2005 – 31 October 2007)

TB = tuberculosis, TB-IRIS = tuberculosis-associated immune reconstitution inflammatory syndrome, MDR-TB = multidrug resistant *M.tb* resistant to rifampin and isoniazid, NTM = Non-tuberculous mycobacteria, CMV = cytomegalovirus, Other *= headache cause undetermined (3), motor axonopathy cause undetermined (2), cerebrovascular accident (1), nosocomial pneumonia and hypoxic ischaemic brain insult (1), cauda equine lesion cause undetermined (1), sub-therapeutic drug level (1)

Table 6.2 summarizes details of the 23 patients with neurologic TB-IRIS. Cerebrospinal fluid analysis for the 13 patients diagnosed with meningitis revealed the following: median lymphocyte count, 30 cells/ μ L (IQR, 14–65 cells/ μ L); median polymorph count, 0 cells/ μ L (IQR, 0–5 polymorphs/ μ L); median protein level, 1.6 g/L (IQR, 1.0–2.3 g/L); and median glucose level, 2.1 mmol/L (IQR, 1.7–2.5 mmol/L).

For 21 of 23 patients, we performed computed tomography or magnetic resonance imaging of the brain or spinal cord. Space-occupying lesions were found in 13 of 21 patients who underwent CNS imaging. Immunoglobulin G (IgG) serologic analysis for *Toxoplasma* species was performed for 11 of these 13 patients, 7 of whom had positive results; however, all 7 had radiologic features of tuberculosis outside of the nervous system. We treated 7 patients with therapeutic trimethoprim-sulfamethoxazole (320 mg/1600 mg twice daily) to cover possible cerebral toxoplasmosis; 4 of these 7 patients had IgG test results positive for *Toxoplasma* species.

Six patients (patients 7, 8, 10, 11, 18, and 21) had evidence of neurologic tuberculosis before ART initiation. The remaining patients did not have clinical features of neurologic tuberculosis before ART initiation. At neurologic deterioration, we sent 17 patients' CNS specimens for mycobacterial culture; 16 had negative results, and 1 patient's culture grew *M.tb* (patient 20).

Twenty (87%) of 23 patients required hospital admission (median duration, 12 days; IQR, 6–24 days), 21 (91%) received corticosteroids (median duration of therapy, 58 days; IQR, 29– 86 days), and 7 (30%) received intensified antituberculosis treatment. Initial clinical improvement occurred in 19 (83%) of the 23 patients; 18 of 19 patients with initial improvement received corticosteroids. The median duration from corticosteroid treatment to initial clinical

improvement was 10 days (IQR, 4–22 days). Three (13%) of 23 patients died within 6 months after initial assessment for neurologic deterioration. Four (17%) of 23 patients were lost to follow-up 6 months after the initial assessment for neurologic deterioration. In these 4 patients, duration from initial assessment to loss to follow-up (presumed dead) ranged from 121 to 278 days. Known survival was thus 70% at 6 months after neurologic TB-IRIS diagnosis. We documented full physical and mental recovery in 10 (63%) of 16 patients who were alive and under our care at 6 months. Six (37%) of 16 patients had residual neurologic deficits at 6 months after initial presentation.

Below, we describe case reports of tuberculoma IRIS, tuberculous meningitis IRIS, and TB-IRIS spondylitis with radiculopathy, which occurred in patients 12, 14, and 23, respectively, from tables 6.1 and 6.2.

Table 6.1: Baseline characteristics, TB – HIV-1 data, and details of neurological

Case	Sex	Age	Previous TB	Site of TB prior to ART	Initial TB Result, (site obtained)	Duration TB treatment to ART (weeks)
1	M	43	No	Nodal	AFB visualised, (node + sputum)	7
2	F	34	Yes	Pleural	No microbiological confirmation	8
3	M	44	No	Disseminated	<i>M. tuberculosis</i> cultured, (node)	4
4	M	35	Yes	Pleural	<i>M. tuberculosis</i> cultured, (sputum)	12
5	F	24	No	Disseminated	<i>M. tuberculosis</i> cultured, (node)	4
6	F	39	No	Pulmonary	No microbiological confirmation	11
7	F	28	Yes	Disseminated	<i>M. tuberculosis</i> cultured, (sputum)	19
8	F	23	Yes	Disseminated	<i>M. tuberculosis</i> cultured, (CSF)	9
9	F	37	No	Disseminated	No microbiological confirmation	5
10	F	43	No	Meningitis, SOL	AFB visualised, (CSF)	35
11	F	30	No	Meningitis	No microbiological confirmation	13
12	F	36	No	Disseminated	AFB visualised, (sputum)	19
13	F	30	Yes	Abdominal nodes	No microbiological confirmation	5
14	M	34	No	Disseminated	No microbiological confirmation	2
15	F	33	No	Disseminated	No microbiological confirmation	10
16	F	28	No	Disseminated	<i>M. tuberculosis</i> cultured, (sputum)	13
17	M	37	Yes	Disseminated	AFB visualised, (sputum)	7
18	M	30	No	Meningitis	No microbiological confirmation	12
19	M	27	Yes	Disseminated	AFB visualised, (sputum)	2
20	M	31	No	Pulmonary	AFB visualised (sputum)	7
21	F	41	No	Meningitis	No microbiological confirmation	10
22	F	26	Yes	Disseminated	<i>M. tuberculosis</i> cultured, (sputum)	6
23	F	31	No	Disseminated	<i>M. tuberculosis</i> cultured, (sputum)	34
Percentage (%)	M: 35		No: 65			
Median		33				9
IQR		28-37				6-13

Key: TB = tuberculosis, TB-IRIS = TB immune reconstitution inflammatory syndrome, ART = combination antiretroviral
 All patients received D4T/3TC/EFV antiretroviral treatment except cases 16, 17 and 21 who received AZT/3TC/EFV and
 EFV = efavirenz 600mg nocte, AZT = zidovudine 300mg twice daily,

symptoms for HIV-1 infected patients with paradoxical neurological TB-IRIS (n=23)

Duration ART to TB-IRIS (days)	CD4+ Pre-ART	CD4+ Post-ART	Duration ART initiation to CD4+ (days)	Neurological symptoms and signs
14	89	260	178	Headache, cognitive impairment, left arm weakness
9	25	-	-	Confusion, fever
19	134	502	224	Headache and focal seizures of right arm
11	139	423	151	Seizures and left hemiparesis
3	12	6	23	Generalized tonic clonic seizures and post-ictal confusion
28	79	326	306	Sudden onset right arm and leg weakness with sensory loss
2	33	-	-	Seizures and confusion
49	254	1043	214	Headache and generalized tonic clonic seizures
33	17	-	-	Headache, left arm and leg seizures, confusion
21	3	-	-	Headache, neck stiffness, confusion and fever
4	95	423	187	Headache, fever, neck stiffness, generalized tonic clonic seizures
16	50	-	-	Headache, neck stiffness, vomiting and hysteria
5	19	104	157	Headache, confusion, neck stiffness, photophobia and vomiting
1	77	207	173	Headache, fever, vomiting
1	61	539	359	Headache, nausea, vomiting and night sweats
8	37	118	110	Headache, fever and neck stiffness
1	8	111	221	Neck stiffness, confusion, generalized tonic clonic seizures
30	158	-	-	Headache, fever, vomiting and neck stiffness
38	48	-	-	Headache, neck stiffness, fever
14	52	159	301	Headache, neck stiffness
30	80	-	-	Headache and worsening lower limb weakness
7	137	-	-	Headache and lower limb weakness, incontinence of urine + faeces
61	99	703	173	Right leg weakness, lower back and right hip pain
14	61	293	183	
5-29	29-97	128-482	161-223	

therapy, AFB = acid fast bacilli, SOL = space-occupying lesion, CSF = cerebrospinal fluid, IQR = interquartile range, case 10 who received D4T/3TC/NVP, D4T= stavudine 30mg twice daily, 3TC = lamivudine 150mg twice daily, NVP = nevirapine 200mg twice daily

Table 6.2: Neurological disease category, investigations, management and outcome

Case	Neurological TB-IRIS category	Cerebrospinal fluid				Brain/ spinal imaging	TB result at neurological deterioration	TB regimen at neurological deterioration
		L	P	Pr	Glc			
1	SOL	-	-	-	-	CT brain: 1 SOL	AFB visualised, (node)	HRZE
2	SOL	0	0	1.6	3.1	CT brain: 1 SOL	Culture negative (CSF)	HRZE
3	SOL	0	0	0.3	3	CT brain: multiple SOL	No culture (CSF)	HR
4	SOL	-	-	-	-	CT brain: SOL	No specimen obtained	HRE
5	SOL	0	0	0.4	3.1	CT brain: 4 SOL	Culture negative (CSF)	HRZE
6	SOL	-	-	-	-	CT brain: 1 SOL	Culture negative (sputum)	HR
7	SOL	3	0	0.9	2.1	MRI brain: 1 SOL	Culture negative (CSF)	HR
8	SOL + Meningitis	8	3	0.8	NP	CT brain: 3 SOL	No culture (CSF)	HRZE
9	SOL + Meningitis	29	0	1.3	4.1	CT brain: 3 SOL	Culture negative (CSF)	HRZE
10	SOL + Meningitis	2	0	2.3	2	CT brain: 3 SOL	No culture (CSF)	HR
11	SOL + Meningitis	72	0	1.8	2	CT brain: 1 SOL	Culture negative (CSF)	HR
12	SOL + Meningitis	16	0	2.3	0.7	CT brain: 1 SOL	Culture negative (CSF)	HR
13	Meningitis	45	0	1.1	1.5	CT brain: atrophy	Culture negative (CSF)	HRZES
14	Meningitis	35	8	1.4	2.6	NP	Culture negative (CSF)	HRZE
15	Meningitis	28	74	0.7	1.6	CT brain: basal ganglia infarct	Culture negative (CSF)	HR
16	Meningitis	25	31	5.1	2	CT brain: multiple SOL	Culture negative (CSF)	HRZE
17	Meningitis	13	6	0.7	2.3	CT brain: no SOL	Culture negative (CSF)	HRZE
18	Meningitis	14	0	0.9	2.7	CT brain: no SOL	Culture negative (CSF)	HR
19	Meningitis	5	2	1.6	0.4	CT brain: Meningeal enhancement, 1 infarct	Culture negative (CSF)	HRZES
20	Meningitis	30	0	2.3	2.2	CT brain: no SOL	<i>M.tb</i> cultured (CSF)	HR
21	Radiculomyelitis	-	-	23	2.9	NP	Culture negative (CSF)	HRZE
22	Radiculomyelitis	86	0	72	2.3	MRI spine: radiculomyelitis	Culture negative (CSF)	HRZE
23	Spondylitis with radiculopathy	-	-	-	-	MRI spine: spondylitis	Culture negative (aspirate)	HR

Key: TB = tuberculosis, TB-IRIS = TB immune reconstitution inflammatory syndrome, ART = combination antiretroviral therapy, cell/ μ L, polymorphonuclear cells (< 1 cell/ μ L), protein (0.15 – 0.45g/L), glucose (2.2 – 3.9mmol/L), CT = computerised Isoniazid, P = Pyrazinamide, E = Ethambutol, S = Streptomycin, TMP-SMX = trimethoprim sulfamethoxazole, SOL = space-outcome = outcome 6 months after neurological deterioration, LTF = lost to follow-up 6 months after neurological deterioration,

of HIV-1 infected patients with paradoxical neurological TB-IRIS (n=23)

TB treatment intensified	Cortico-steroids prescribed, duration (weeks)	ART stopped	TMP-SMX chemo-prophylaxis prior to deterioration (160/800mg daily)	Toxo-plasma serology (IgG)	Therapeutic TMP-SMX (320/1600mg twice daily) after deteriorat'n	Syphilis serology, (site)	6-month outcome
No	Yes, 10*	No	Yes	Pos	No	Pos (blood)	Alive, #
Yes	Yes, 5	Yes	Yes	Neg	Yes	NP	Died
Yes	Yes, 49*	Yes	Yes	Neg	Yes	Neg (blood)	Alive
No	Yes, 13*	No	Yes	Neg	Yes	Neg (blood)	Alive
No	Yes, 17*	No	Yes	Pos	Yes	NP	Alive, #
No	No*	No	NA	Pos	Yes	Neg (blood)	Alive, #
Yes	Yes, 4*	No	Yes	Pos	Yes	Neg (blood)	LTF
No	Yes, 11*	No	Yes	Pos	No	Neg (blood)	Alive, #
No	Yes, 4*	No	NA	Neg	No	Neg (CSF)	Alive
Yes	Yes, 1	Yes	Yes	Pos	Yes	Neg (CSF)	Died
Yes	Yes, 1*	No	NA	NP	No	NP	Alive, #
Yes	Yes, 8*	No	Yes	Pos	No	Neg (CSF)	Alive
No	Yes, 1*	No	Yes	NP	No	Neg (blood)	Alive
No	Yes, 2*	No	Yes	NP	No	Neg (blood)	Alive, #
No	Yes, 19*	No	Yes	NP	No	Neg (blood)	Alive, #
No	Yes, 10*	No	Yes	NP	No	Neg (blood)	Alive
No	Yes, 1*	No	Yes	NP	No	Neg (blood)	Alive, #
No	Yes, 1*	No	NA	NP	No	Neg (blood)	LTF, #
No	Yes, 6*	No	Yes	NP	No	Neg (CSF)	Died
Yes	Yes, 11*	No	Yes	NP	No	Neg (CSF)	Alive, #
No	No	No	NA	NP	No	Neg (blood)	LTF
No	Yes, 8	No	NA	NP	No	NP	LTF
No	Yes, 22*	No	NA	NP	No	NP	Alive

L = lymphocytes, P = polymorphonuclear cells, Pr = protein, Glc = glucose, Normal cerebrospinal fluid values: lymphocytes < 5 tomography, MRI = magnetic resonance imaging, *M.tb* = *Mycobacterium tuberculosis*, TB regimens: R = Rifampin, H = occupying lesion, NA = not ascertained, NP = not performed, Neg = negative, Pos = positive, CSF = cerebrospinal fluid, 6-month * = initial clinical improvement, # = full physical and mental recovery

Patient 12: paradoxical brain tuberculoma and meningitis IRIS. A 36-year-old woman with HIV-1 infection received a diagnosis of disseminated tuberculosis. Acid-fast bacilli were seen in her sputum sample, a chest radiograph showed a miliary pattern, and abdominal ultrasound visualized splenic hypo-densities, ascites, and a pericardial effusion. She commenced antituberculosis treatment with isoniazid, rifampin, pyrazinamide, and ethambutol. She gained weight, her cough and sweating improved, and her fever resolved. Her CD4+ cell count nadir was 50 cells/ μ L, and 19 weeks after starting antituberculosis treatment, she initiated ART (stavudine 30 mg twice daily, lamivudine 150 mg twice daily, efavirenz 600 mg nightly). She received trimethoprim-sulfamethoxazole chemoprophylaxis (160 mg/800 mg twice daily). Sixteen days after initiation of ART, she developed headache, neck stiffness, and vomiting. Computed tomography of her brain showed a 10-mm partly solid, partly cystic, inhomogeneously enhancing lesion in the left temporo-parietal area (figure 6.2), with surrounding oedema. Lumbar puncture showed an aseptic lymphocytic meningitis (table 6.2). Results of bacterial and fungal cultures, as well serological tests for syphilis performed on cerebrospinal fluid samples, were negative. Although serological test results were positive for toxoplasmosis, the patient did not receive therapeutic trimethoprim-sulfamethoxazole (320 mg/1600 mg twice daily). We intensified antituberculosis treatment from isoniazid and rifampin to isoniazid, rifampin, pyrazinamide, and ethambutol, and we prescribed prednisone (60 mg daily; 1.5 mg/kg/day) for 4 weeks, followed by a 4-week taper. The patient's headache and neck stiffness resolved. No subsequent deterioration occurred.



Figure 6.2: Paradoxical brain tuberculoma (arrowed) and meningitis IRIS

Computed tomography showing a 10-mm partly solid, partly cystic, non-homogeneously enhancing lesion in the left temporo-parietal area

Patient 14: paradoxical TB-IRIS meningitis. A 33-year old man with HIV-1 infection received a diagnosis of miliary tuberculosis on the basis of chest radiograph findings. We were unable to obtain microbiological confirmation of tuberculosis, because sputum induction was unsuccessful. The patient had no history of tuberculosis and started treatment with isoniazid, rifampin, pyrazinamide, and ethambutol. He experienced improvement while receiving antituberculosis treatment; cough, shortness of breath, and generalized weakness resolved. Sixteen days after starting antituberculosis treatment, he initiated ART (stavudine, lamivudine, and efavirenz), because his CD4⁺ cell count nadir was 41 cells/ μ L. One day after initiation of ART, the patient developed headache, fever, and vomiting. On physical examination, he was febrile and tachycardic (heart rate, 128 beats per min) but had no nuchal rigidity. We performed a lumbar puncture, which revealed an aseptic lymphocytic meningitis (table 2). The patient continued to receive antituberculosis treatment with isoniazid and rifampin and received prednisone (40 mg twice daily) for 16 days. His headache, fever, and vomiting resolved, and he experienced no subsequent deterioration.

Patient 23: Paradoxical TB-IRIS spondylitis complicated by radiculopathy. A 31-year old HIV-1 infected woman was diagnosed with disseminated tuberculosis; she had constitutional symptoms, abdominal ultrasound showed ascites and adenopathy and we cultured *Mycobacterium tuberculosis* sensitive to isoniazid and rifampin from her sputum. She had no previous history of tuberculosis and commenced isoniazid, rifampin, pyrazinamide, and ethambutol. She stopped coughing, she gained weight and her malaise and night sweats resolved. Her CD4⁺ count nadir was 99 cells/ μ L, and 24 weeks after starting antitubercular treatment she initiated ART – (stavudine, lamivudine, and efavirenz). Ten days after ART initiation, her symptoms of night sweats and anorexia recurred and chest radiograph showed a new miliary infiltrate. 61 days after ART initiation, she developed back-pain radiating down

her right leg. On examination, we found weakness of hip flexion and knee extension, loss of the right knee reflex but no sensory deficit; her clinical features were in keeping with a right L3/4 radiculopathy. Magnetic resonance imaging of her spine confirmed tuberculous spondylitis with an epidural component impinging the right aspect of the thecal sac (arrowed black). We diagnosed a neurological manifestation of paradoxical TB-IRIS and she started prednisone 60mg daily (1.5mg/kg/day). Despite prednisone, her pain persisted. We performed a CT guided paraspinal fine needle aspirate (arrowed white), which on microscopy showed 1+ neutrophils, and was culture negative for pyogenic bacteria, mycobacteria and fungi. She received a total of 152 days of corticosteroids, which we tapered on cessation. Her back pain improved with opiate and non-steroidal anti-inflammatory drugs, but she had residual weakness and sensory loss affecting the lateral aspect of her right thigh. 173 days after ART initiation, her CD4+ count had risen to 700 cells/ μ L, her viral load was 500 copies/ mL.



Figure 6.3: Paradoxical TB-IRIS spondylitis complicated by radiculopathy.

Magnetic resonance imaging of the spine showing tuberculous spondylitis (arrowed black) with an epidural component impinging the right aspect of the thecal sac, as well as a paravertebral collection (arrowed white).

6.4 Discussion

Our study is, to our knowledge, the first case series of neurologic TB-IRIS. We found that neurologic TB-IRIS accounts for 10% of paradoxical TB-IRIS cases in a hospital setting. We found a mortality rate of 13% (3 deaths among 23 patients) for neurologic TB-IRIS, but the mortality rate may have been 30% (7 deaths among 23 patients) if all 4 patients who were lost to follow-up died. Published data suggest that, in developing countries, many patients in antiretroviral programs who are lost to follow-up actually die (Braitstein 2006). In a Vietnamese report that described 44 patients with HIV-1 infection who received dexamethasone for tuberculous meningitis, 27 (61%) of 44 patients died (Thwaites 2004). Reasons for differences in mortality rates include non-availability of ART in the Vietnamese study and the fact that the Vietnamese study assessed outcome at 9 months, not 6 months. In addition, in our study, by analyzing data for patients who were referred with TB-IRIS, we may have selected for a group of patients with neurologic tuberculosis who had a more favourable prognosis. Patients with more-severe and more-extensive neurologic tuberculosis may have died before receiving ART. Also, patients who developed neurologic TB-IRIS may have died before referral to our hospital. The published literature reports only 1 death due to neurologic TB-IRIS (Burman 2007); however, this is almost certainly because of a paucity of published case reports (Lee 2007, Dautremer 2007, Sumner 2003, and Vidal 2003).

In our study, initial improvement of symptoms and/or signs occurred in 19 of 23 patients; 18 of these 19 patients with initial improvement received corticosteroids. Full physical and mental improvement occurred in 10 of 16 patients who were alive and in care at 6 months. A substantial number of patients (6 of 16 patients) had residual neurologic disability at 6 months that was likely to be permanent. A recent meta-analysis of published literature does not support or refute the use of corticosteroids to reduce death and neurologic deficit among patients with

HIV-1 infection who have tuberculous meningitis (Prasad 2008). Corticosteroids are proposed as a treatment for TB-IRIS, although available evidence is anecdotal (Lawn 2007, Lesho 2006). To date, only one clinical trial supports the use of corticosteroids in treating non-life-threatening TB-IRIS (Meintjes 2009). Corticosteroids reduced duration of hospital admission and improved symptoms. We are unable to conclude from our observational study whether corticosteroids reduce mortality or disability in neurologic TB-IRIS.

To diagnose TB-IRIS, differential diagnoses need to be excluded or resolution should occur without treatment for other opportunistic infections (Meintjes 2009). This is difficult in cases involving space-occupying lesions because of limited access to brain biopsy. Differential diagnoses for TB-IRIS include cryptococcoma, progressive multifocal leukoencephalopathy IRIS (Torok 2008), bacterial abscess, lymphoma, neurocysticerci, and syphilitic gumma. We were unable to definitively exclude all other differential diagnoses by means of brain biopsy; however, 10 of 12 patients with space-occupying lesions did not experience deterioration despite receiving no treatment for these other aforementioned illnesses.

The major differential diagnosis for tuberculoma IRIS, however, is cerebral toxoplasmosis. A subgroup of patients (7 of 12 patients) received simultaneous treatment for toxoplasmosis and tuberculosis in our study. In certain parts of South Africa, *Toxoplasma gondii* is isolated more commonly than *M.tb* from biopsy specimens and aspirates of intracranial mass lesions (Bhigjee 1999), whereas in other parts of South Africa, *M.tb* is isolated more commonly (Modi 2004). We anticipate a higher proportion of tuberculosis cases in South Africa, because the tuberculosis case notification rate in Cape Town is one of the highest in the world (Harrison 2007). In addition, key features that suggest tuberculosis, rather than other infectious causes, include (1) concurrent presence of pulmonary tuberculosis or other non-neurologic tuberculosis

and (2) basal meningeal enhancement (Modi 2004). All of the patients in our study whose tuberculosis diagnosis was microbiologically unconfirmed had radiologic features of non-neurologic tuberculosis. In addition, of 7 patients who were treated for possible cerebral toxoplasmosis, 3 had serologic test results that were negative for toxoplasmosis, and all 7 had radiologic evidence of non-neurologic tuberculosis. Although we had strong clinical suspicion of paradoxical neurologic TB-IRIS in these 7 patients, we opted to also treat for possible cerebral toxoplasmosis, because the clinical condition of these patients was severe, and a confirmatory diagnostic test for TB-IRIS is not yet available. Trimethoprim-sulfamethoxazole, used in our setting, and pyrimethamine-sulfadiazine, used elsewhere, have no significant difference with respect to clinical efficacy during short-term therapy for toxoplasmic encephalitis (Torre 1998).

Patients without apparent neurologic tuberculosis before ART may present with neurologic TB-IRIS after ART initiation. This occurred in 17 of 23 patients in our case series. Presumably, this is attributable to subclinical seeding of *M. tuberculosis* into neurologic tissue that provokes an inflammatory response at the time of IRIS. Although mycobacterial culture results are usually negative at TB-IRIS diagnosis, *M. tuberculosis* may be cultured, particularly if IRIS occurs early during antituberculosis treatment (Meintjes 2008). This occurred in 1 patient.

Incident MDR tuberculosis may occur in $\approx 10\%$ of HIV-1 infected patients with tuberculous meningitis (Torre 1998). Patients with MDR tuberculous meningitis have significantly poorer outcomes (Thwaites 2005). Previously, we diagnosed rifampin-resistant tuberculosis in $>10\%$ of patients with suspected TB-IRIS (Meintjes 2009). In this study, we diagnosed MDR tuberculosis in $\approx 10\%$ of patients with suspected neurologic TB-IRIS. We empirically intensified antituberculosis treatment in 7 patients with paradoxical neurologic TB-IRIS while

awaiting mycobacterial culture and drug susceptibility results; however, none of these patients cultured MDR tuberculosis. We are unable to ascertain the extent that intensified antituberculosis treatment improved clinical outcome. It is possible that the 7 patients who received a diagnosis of HIV-1 encephalopathy and delirium (figure 1) may have experienced cognitive deterioration as a result of cerebral inflammation (cerebritis) due to TB-IRIS. However, in these patients, we did not diagnose neurologic TB-IRIS, because either (1) the patient did not have a space-occupying lesion and thus did not fulfil our case definition, or (2) the imaging modality that we used (computed tomography rather than magnetic resonance imaging in most cases) was not sensitive enough to detect all space-occupying lesions (Wasay 2003, Morgado 2005). This may be a limitation of our case definition and warrants further investigation.

We report a median duration of 14 days from ART initiation to the onset of TB-IRIS symptoms in both this study and our previous study of paradoxical TB-IRIS (Meintjes 2009). Other studies report a median duration from ART initiation to the onset of TB-IRIS symptoms of 12 days (IQR, 5–17 days, Narita 1998), 34 days (IQR, 8–97 days, Burman 2007), and 47 days (IQR, 36–81 days, Kumarasamy 2004). Variability in baseline CD4+ cell counts and duration of antituberculosis treatment before ART initiation may account for the discrepancy among studies, because these are risk factors for TB-IRIS (Michailidis 2005, Shelburne 2005). The median duration of hospitalization in patients with TB-IRIS is usually 1 week (Burman 2007), which is similar to our findings. In HIV-1–infected patients with tuberculous meningitis, the median symptom duration before assessment varies from 11 to 18 days (Katrak 2000, Thwaites 2005, Torok 2008). Our shorter median symptom duration of 6 days before assessment for neurologic TB-IRIS may indicate increased inflammation from IRIS. Differing referral patterns between our setting and other settings may also account for the shorter symptom duration.

In a hospital setting, patients with suspected non-neurologic TB-IRIS are more likely than patients with suspected neurologic TB-IRIS to receive an eventual diagnosis of TB-IRIS. We attribute this finding to screening of patients by physicians at primary care clinics. It is likely that patients with suspected non-neurologic TB-IRIS with alternative illnesses to TB-IRIS were not assessed at our hospital, because they improved with appropriate treatment prescribed by primary care physicians. Intuitively, patients with suspected TB-IRIS with neurologic deterioration require rapid referral to the hospital for inpatient admission and investigation, thus increasing the likelihood of diagnosis of other illnesses.

Although paradoxical neurologic TB-IRIS is a potentially life threatening manifestation of paradoxical TB-IRIS, there is most likely a spectrum of disease severity in neurologic TB-IRIS. The milder forms of neurologic TB-IRIS with spontaneous recovery, and the extremely severe neurologic TB-IRIS with rapid progression to death before referral, may not have been included in our case series. We did not microbiologically confirm tuberculosis in 9 (39%) of 23 patients; however, tuberculosis is difficult to confirm microbiologically in neurological disease. *M.tb* was cultured from only 31% of patients with tuberculous meningitis in a Vietnamese study (Thwaites 2004). All 3 patients who died with a diagnosis of paradoxical neurologic TB-IRIS did not have cultures positive for *M. tuberculosis*; thus, drug susceptibility testing to exclude MDR tuberculosis could not be performed. We obtained cerebrospinal fluid for analysis from 18 of 23 patients but could have improved diagnostic accuracy by performing autopsies and brain biopsies. Brain biopsies carry the risk of postoperative haemorrhage (Nicolato 1997) and are difficult to access in our setting. Standardized tools were not used to assess neurocognitive function or neurologic outcomes. Rather, we relied on the bedside clinical assessment of the attending physician at hospital admission and discharge, as well as

records from clinic follow-up. Lastly, this study was conducted at a referral hospital that served a large population with a high incidence of HIV-1–associated tuberculosis. Our findings may not be generalizable to primary care settings or settings where HIV-1–associated tuberculosis is less common.

In conclusion, in a hospital setting, neurologic TB-IRIS is a not-infrequent manifestation of TB-IRIS and causes considerable short-term morbidity but is associated with reasonable long-term outcomes. Future prospective studies may better (1) determine the incidence of neurologic TB-IRIS among patients with initial neurologic and non-neurologic tuberculosis disease; (2) determine optimal management strategies to reduce death, neurologic deficits, and disabilities; and (3) describe the pathophysiology of neurologic TB-IRIS.

University of Cape Town

Chapter 7

Conclusions

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7.1 Key findings and future work

This thesis focused on various issues relating to clinical deterioration during antituberculosis treatment in a high HIV-1 prevalence setting. We report the following findings:

Firstly, clinical deterioration is frequently encountered in our setting. At the tuberculosis clinic (Khayelitsha Site B), up to 40% of patients experienced clinical deterioration within 24 weeks of commencing antituberculosis treatment. At the referral hospital (GF Jooste Hospital), clinical deterioration accounted for 17% of medical inpatient admissions. The most significant risk factor for clinical deterioration was profound immune-suppression due to HIV-1 infection.

Secondly, the causes for clinical deterioration in HIV-1 infected patients at the referral hospital and at the tuberculosis clinic are similar (table 7.1); the baseline characteristics and the proportion of patients who died are also similar. The median interval from commencing antituberculosis to clinical deterioration differed: this is likely related to study design. The clinic-based study followed patients for the first 24 weeks from initiation of antituberculosis treatment, whereas the hospital-based study enrolled patients presenting over a 3-month period, regardless of duration of antituberculosis treatment. A higher proportion of patients in the clinic-based study developed deep venous thrombosis. This may reflect better clinical

ascertainment of venous thrombosis in the clinic based study, but does not explain why this difference only occurred with the diagnosis of venous thrombosis. Thirdly, drug resistance is an important but infrequent cause of clinical deterioration. Drug resistant *M.tb* occurred in 4-10% of HIV-1 infected patients with clinical deterioration; 37% of these patients died within 6 months of clinical deterioration.

Table 7.1: Comparison of HIV-1 infected patients who experienced clinical deterioration in the hospital based (n=291) and clinic based (n=101) studies			
	Hospital based (n=291)	Clinic based (n=101)	p-value
Female, n (%)	153 (53)	45 (54)	0.168
Age in years, median (IQR)	34 (28 – 41)	36 (29 – 43)	0.051
Previous TB, n (%)	101 (35)	31 (31)	0.394
Median CD4 count (cells/mm³), n (IQR)	82 (38 – 157)	79 (33 - 199)	0.579
Extra-pulmonary TB, n (%)	141 (48)	46 (46)	0.645
Microbiologic confirmation at TB diagnosis¹, n (%)	134 (46)	57 (56)	0.083
Microbiologic confirmation with drug susceptibilities known at TB diagnosis¹, n (%)	85 (29)	29 (29)	0.621
TMP-SMX² chemoprophylaxis, n (%)	-	91 (90)	-
Interval from TB treatment to clinical deterioration, n (IQR)	93 (44 - 155)	50 (20 - 88)	<0.001
Receiving ART at clinical deterioration, n (%)	122 (48)	31 (30)	0.058
Diagnoses at clinical deterioration, n (%)			
MDR-TB	30 (10)	4 (4)	0.063
TB-IRIS	51 (18)	22 (22)	0.344
Paradoxical reaction	31 (11)	17 (17)	0.114
AIDS	80 (27)	21 (21)	0.234
Bacterial infection	32 (11)	9 (9)	0.706
Gastroenteritis	37 (13)	8 (8)	0.211
Drug side-effects	35 (12)	14 (14)	0.605
Deep vein thrombosis	12 (4)	10 (10)	0.042
Death	43 (15)	15 (15)	1.000
Key TB: tuberculosis, TMP-SMX: trimethoprim sulfamethoxazole (160/800mg), ART: antiretroviral treatment, MDR-TB: <i>Mycobacterium tuberculosis</i> resistant to rifampin and isoniazid, TB-IRIS: tuberculosis-associated immune reconstitution inflammatory syndrome AIDS: Acquired Immune Deficiency Syndrome IQR: interquartile range, p-value significant at < 0.05			

Drug resistant bacterial infections, caused by organisms such as ESBL and MRSA, only occurred in the first hospital study; 75% (9/12) of these patients died within 11 days of obtaining the specimen. We attribute the absence of ESBL and MRSA organisms in the second study to improved infection control measures and antibiotic policies as cultures were requested on all specimens obtained during hospitalisation in the clinic-based study. Also, ESBL and MRSA organisms appear less common in the community setting, strengthening the conclusion that these were hospital acquired bacterial infections.

Fourthly, the poor outcome of HIV-1 infected patients at 24 weeks is very concerning: 22% of patients were lost to follow-up, 8% of patients died, and 31% of patients eligible for ART at tuberculosis diagnosis did not receive ART within 24 weeks. The reasons for loss to follow-up need to be determined in future prospective studies. While a major breakthrough has been achieved in now being able to offer ART to tuberculosis patients with CD4+ counts < 350 cells/ μ L, this breakthrough is not without its own challenges. The benefits of ART initiation at higher CD4+ counts requires major changes in current medical infrastructure: a major paradigm shift in the South African public health care sector is still needed. The current reactionary approach needs to be replaced with a pro-active, investigatory approach, encouraging continuity of care and patient follow-up. Medical information systems require further development and improvements to allow consistent access to patients' health information across districts and provinces.

Fifthly, TB-IRIS is a rising phenomenon in our setting. TB-IRIS accounts for approximately 20% of the reasons for clinical deterioration during antituberculosis treatment, both in the clinic and hospital setting. In the hospital setting, neurologic TB-IRIS is a not infrequent manifestation of TB-IRIS and causes considerable short-term morbidity. Numerous potential avenues of research exist, as future prospective studies are required to determine the

pathophysiology of TB-IRIS. The temporal sequence of events in paradoxical TB-IRIS may prove crucial. Focusing on the intervals between tuberculosis diagnosis, ART initiation and TB-IRIS occurrence may better define the pathophysiology of TB-IRIS. Figure 7.1 describes the temporal sequence of events leading to TB-IRIS. Furthermore, cerebrospinal cytokine analysis, proteomics and metabolomics may offer invaluable insights regarding TB-IRIS meningitis. Magnetic resonance imaging, single photon emission computed tomography (SPECT) scanning and brain biopsies may prove invaluable in understanding TB-IRIS tuberculomata. These studies are already underway in Cape Town. We have also observed an interesting relationship between TB-IRIS and paradoxical reactions; figure 7.2 reports a patient who developed both conditions in succession. We are also investigating whether particular lineages of *M.tb* are associated with TB-IRIS. Lastly, we are investigating whether sub-therapeutic rifampin drug levels are an important cause of clinical deterioration in patients referred to the hospital.

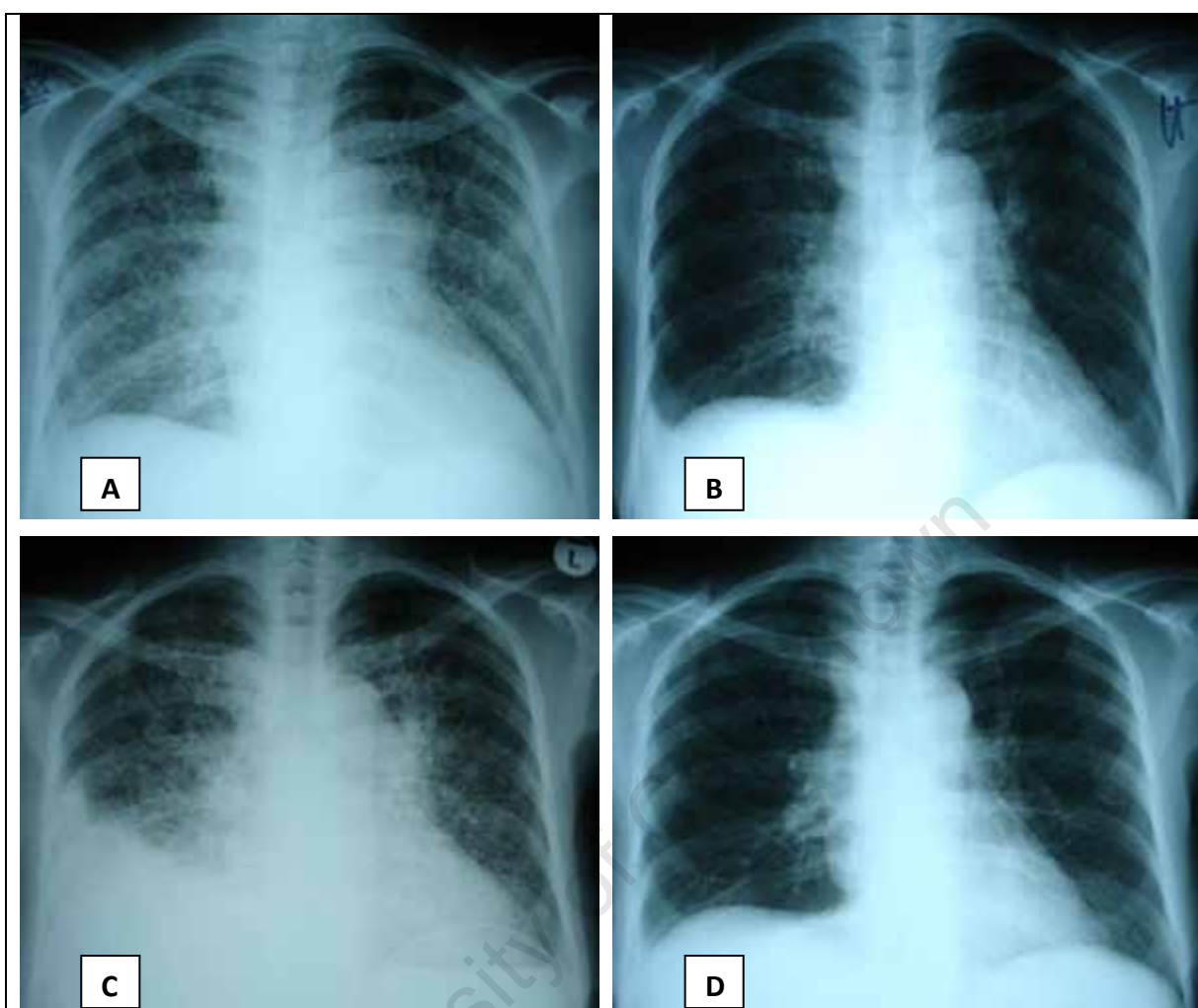


Figure 7.1: Paradoxical pulmonary and pleural TB-IRIS treated with prednisone

A 43 year-old HIV-infected woman was diagnosed with disseminated tuberculosis. An aspirate from a cervical node cultured *Mycobacterium tuberculosis* (susceptible to rifampicin and isoniazid) and her chest radiograph showed a bilateral reticulonodular infiltrate with large hilar adenopathy (A). She commenced antitubercular treatment – isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E). Her cough resolved, and her night sweats and appetite improved. Chest radiography also improved (B). Her CD4 count nadir was 20 cells/ μL . Ten weeks after starting antitubercular treatment she initiated combination antiretroviral treatment (ART) - stavudine (D4T) 30mg twice daily, lamivudine (3TC) 150mg twice daily, efavirenz (EFV) 600mg nocte. Eight days later, she developed shortness of breath, and recurrence of her cough. Her CD4 count had increased to 64 cells/ μL . Chest radiography at TB-IRIS showed a new right pleural effusion and recurrence of pulmonary infiltrate (C). We drained 750ml of straw-coloured pleural fluid, which did not culture mycobacteria. We continued HRZE and ART and prescribed prednisone 70mg daily (1.5mg/kg/day) for 4 weeks. Her cough and night sweats resolved. Chest radiography showed dramatic improvement (D). She, however, developed cytomegalovirus retinitis of her left eye. Prednisone was stopped and she was treated with intravitreal ganciclovir with improvement in vision. Six months after ART initiation, her CD4 count was 263 cells/ μL and HIV-1 viral load undetectable.

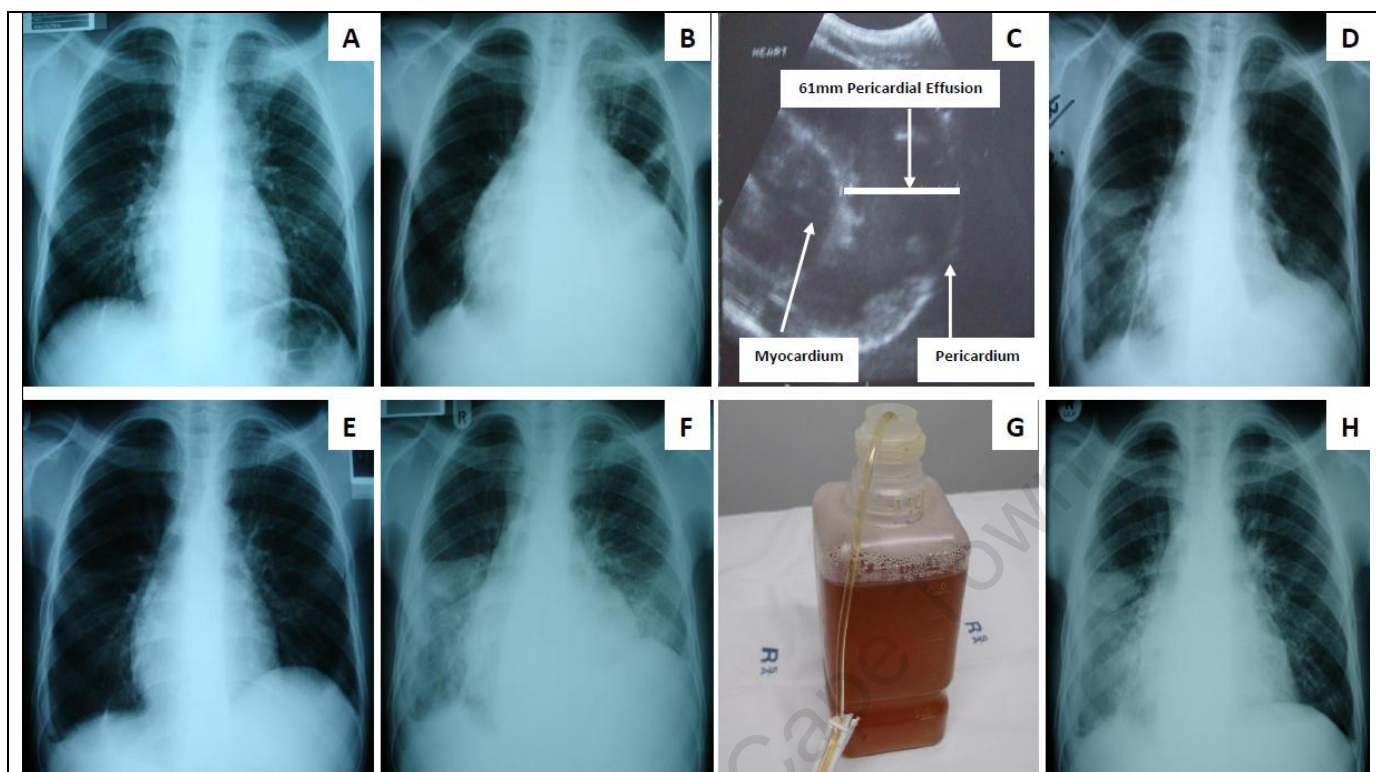


Figure 7.2: Paradoxical reaction and paradoxical TB-IRIS

A 26-year-old HIV-infected man was diagnosed with recurrent pulmonary tuberculosis. His sputum cultured *Mycobacterium tuberculosis* (sensitive to rifampin and isoniazid) and his chest radiograph showed a left apical infiltrate (A). He commenced antituberculosis treatment – isoniazid (H), rifampin (R), pyrazinamide, ethambutol (E) and streptomycin. His cough, fever, nausea and appetite improved. Ten days after starting antituberculosis treatment, he developed shortness of breath on exertion (50 metres), as well as orthopnea. He had features in keeping with a pericardial effusion; his jugular venous pressure was raised at 10cm H₂O, cardiac sounds were muffled, and pulsus paradoxus was present (> 10mmHg). Chest radiography showed cardiomegaly (B). His cardiac ultrasound (C) confirmed a 61mm pericardial effusion with stranding and debris. 1200ml of straw-coloured pericardial fluid was aspirated. He had dramatic improvement immediately after aspiration; he could walk 500 metres, orthopnea resolved and chest radiography improved (D). He also received prednisone for 4 weeks (1.5mg/kg/day for 2 weeks, and 0.75mg/kg/day for 2 weeks). Chest radiography improved further (E). His CD4⁺ count nadir was 5 cells/ μ L. Ten weeks after starting antituberculosis treatment, he initiated combination antiretroviral treatment (ART) - stavudine (D4T) 30mg twice daily, lamivudine (3TC) 150mg twice daily, efavirenz (EFV) 600mg nocte. Ten weeks later, he developed shortness of breath, and recurrence of his cough and fever. His CD4⁺ count had increased to 97 cells/ μ L. Chest radiography at TB-IRIS showed a large left pleural effusion and worsening right pulmonary infiltrates (F). We percutaneously drained 850ml of straw-coloured pleural fluid (G), which did not culture mycobacteria. We continued antituberculosis treatment (HRE) but did not re-initiate prednisone. His cough, fever and shortness of breath resolved. Chest radiography showed improvement (H) although the pulmonary infiltrates persisted. The left pleural effusion did not recur. Six months after ART initiation, his CD4⁺ count was 119 cells/ μ L and HIV-1 viral load undetectable.

7.2 Drugs on the horizon

In chapter 2, we described the difficulties associated with combining current antituberculosis and antiretroviral treatments. In order to meet the challenges of tuberculosis and HIV-1 co-infection, new antituberculosis and antiretroviral drugs are urgently needed. These new antituberculosis drugs should i) shorten the duration of antituberculosis treatment or significantly reduce the number of doses under DOTS, ii) cure both drug-susceptible and drug-resistant tuberculosis, iii) effectively treat LTBI, iv) limit cross-resistance by utilizing a new site of drug activity, and v) not be metabolised by CYP or induce or inhibit CYP. These factors should limit shared toxicities between antituberculosis and antiretroviral treatment. Furthermore, new antiretroviral drugs are needed in the event that drug-resistant HIV-1 emerges in South Africa. We have already seen that HIV-1 associated immune-suppression is a strong risk factor for clinical deterioration during antituberculosis treatment. If drug resistant HIV-1 were to emerge, serious setbacks would occur in the management of HIV-1 associated tuberculosis.

Since 2000, several novel antituberculosis and antiretroviral drug candidates have been identified (Tables 7.2 – 7.4, Global Alliance 2007, Reeves 2005). Many of these antiretroviral drugs are now licensed by the United States Food and Drug Administration (FDA), but still have to reach the South African public sector.

Table 7.2: Antituberculosis and antiretroviral drugs in development

Tuberculosis	HIV-1
<u>Novel chemical entities</u>	Attachment inhibitors
ATP synthase inhibitor:	Dextran sulphate
Diarylquinoline TMC 207	Heparin
FAS20013	Cyanovirin-N
Cell wall inhibitors:	Cyclotriazadisulfonamide analogues
Nitroimidazole PA-824	PRO 2000
Nitroimidazole OPC-67683	TNX 355
Dipiperidine SQ-609	PRO 542
Translocase I inhibitor	BMS 806
InhA inhibitors	Co-receptor binding inhibitors
Isocitrate lyase inhibitors	CCR5: SCH-D
Protein synthesis inhibitor	Maraviroc
Pyrrole LL-3858	Aplaviroc
Other: Pleuromutilins	TAK 779
<u>Based on existing chemical entities</u>	CXCR4: AMD 070
Fluoroquinolones	Plerixafor
Moxifloxacin	Fusion inhibitors
Gatifloxacin	Tifuvirtide
New quinolones	Non-nucleoside reverse transcriptase inhibitors
Non-fluorinated quinolones	Etravirine
Ethambutol derivative:	Integrase inhibitors
SQ-109	L 731988
Macrolides	L 870810
Thiolactomycin analogues	L 870812
Nitrofuranylamines	Maturation inhibitors
Rifamycin derivatives:	PA 457
Rifalazil	Protease inhibitors
Oxazolidinones:	Darunavir
Linezolid	

Table 7.3: Potential benefits, toxicities and metabolism of new antituberculosis in development

TB drugs in development						
Drug Name	Potential benefits	Potential for treating LTBI	Potential for shortening TB treatment	Metabolism and effect on CYP	Potential toxicities	Other
PA-824 ¹	Bacterio-cidal and -static, No cross resistance, Treats MDR-TB	Yes	Yes/ No (Nuermberger 2006)	No CYP metabolism		Requires activation by mTB F420 factor (Rv3547 enzyme)
OPC-67683 ²	No cross resistance, High MTB specificity, Treats MDR-TB	Yes	Yes	No CYP metabolism		Intracellular activity. Requires activation by Rv3547 enzyme
TMC 207 ³	Novel target limiting cross-resistance, Bacterio-cidal and -static, Treats MDR-TB		Yes	Yes, CYP3A4		Synergistic with other TB meds esp. PZA
Moxifloxacin ⁴	Treats MDR-TB	Yes	Yes	Ciprofloxacin inhibits hepatic microsomal enzymes.	GIT, hypersensitivity, CNS, Hepatic necrosis, interstitial nephritis	Cross-resistance among quinolones
Gatifloxacin ⁵	Treats MDR-TB (weak data)		Yes	Ciprofloxacin inhibits hepatic microsomal enzymes.	As above, dysglycaemia	Cross-resistance among quinolones
SQ-109 ⁶	May treat Ethambutol-resistant strains			CYP2D6 CYP2C19		
¹ Ginsberg 2009a, Ginsberg 2009b, Global Alliance 2007, Nuermberger 2005, Nuermberger 2006 ² Ginsberg 2007, Global Alliance 2007, Matsumoto 2006 ³ Andries 2005, Cole 2005, Diancon 2009, Global Alliance 2007, Rustomjee 2008 ⁴ Ginsberg 2007, Global Alliance 2007, Hu 2003, Park-Wyllie 2006 ⁵ Burman 2006, Dorman 2009, Global Alliance 2007, Hu 2003, Nuermberger 2005, Rustomjee 2008 ⁶ Chen 2006, Ginsberg 2007, Global Alliance 2007						

Table 7.4: Potential benefits, toxicities and metabolism of new antiretroviral drugs recently licensed

Antiretroviral drugs in development				
Drug Name	Potential benefits	CYP metabolism / effect	Potential toxicities	Other
Etravirine ¹	Inhibits replication of wild-type & drug resistant HIV-1		No safety concerns in phase II trials	
Darunavir ²	Retains efficacy against multi-PI-resistant viruses, higher genetic barrier to resistance	Metabolised by CYP		
Maraviroc ³	Active against diverse HIV-1 isolates from different clades, Does not compete with chemokine binding,	Yes, substrate for CYP3A4	Mild/ moderate Headache, dizziness, asthenia, flatulence, rhinitis	Immune modulation, escape mutants, altered viral tropism,
Vicriviroc ⁴	Beneficial in salvage therapy		Poor virological outcomes in naïve patients	
Raltegravir ⁵	Virological benefit in salvage therapy	No (metabolised by glucuronidation)	Similar to placebo	Potential interaction with antibiotics
Elvitegravir ⁵	Efficacy against MDR HIV-1	Metabolised by CYP and glucuronidation, induces CYP3A	Similar to placebo	Potential interaction with antibiotics
¹ Boone 2006 ² de Bethune 2006, Towner 2010 ³ The Pinksheet 2007, Reeves 2005 ⁴ Reeves 2005, Suleiman 2010 ⁵ de Castro 2009, Cooper 2007, Mathias 2006, Pommier 2005, Reeves 2005				

7.2.1 TB drugs in development

PA-824

PA-824 is a nitroimidazo-oxazine. It requires activation by M. tuberculosis F420 factor and inhibits synthesis of cell wall lipids as well as protein synthesis. Phase 1 clinical trials of PA-824 by the Global TB Alliance showed good safety, tolerability, and pharmacokinetics. Of concern are reports that PA-824 causes creatinine levels to rise, possibly by inhibition of renal tubular creatinine secretion. Preliminary studies suggest that PA-824 will be active against MDR-TB and has no cross-resistance with other anti-tubercular drugs. Importantly, it is not metabolised by CYP and does not induce or inhibit CYP. It had similar bacteriostatic efficacy to rifampin and was more efficient than isoniazid or moxifloxacin but less efficient than rifampin and isoniazid in continuation phase therapy in the mouse model (Nuermberger 2005, Nuermberger 2006, Global Alliance 2007, Ginsberg 2009a, Ginsberg 2009b).

OPC-67683

OPC-67683 is a nitroimidazo-oxazole that is similar in structure to PA-824. It inhibits cell wall biosynthesis. Otsuka Pharmaceuticals (Japan) are currently conducting phase 2 clinical trials (Ginsberg 2007). Preclinical studies in rodents and dogs suggest that OPC-67683 could be used in HIV-1/AIDS as it has no effect on CYP. It may also shorten treatment, as a result of synergistic *in vitro* activity with rifampin and pyrazinamide. OPC-67683 is effective against MDR-TB *in vitro* and displayed no cross-resistance to first-line antituberculosis treatment. It also has potential to treat LTBI (Matsumoto 2006, Global Alliance 2007).

TMC207

TMC207 is a diarylquinoline and is also known as Compound J and R207910. It inhibits ATP synthase (AtpE) leading to ATP depletion and pH imbalance (Andries 2005, Cole 2005). It was initially identified by Johnson & Johnson and subsequently developed by the subsidiary Tibotec Pharmaceuticals Limited. It has recently undergone phase 2a clinical trials in drug-sensitive tuberculosis, as the first novel anti-TB compound to be studied in patients in nearly 4 decades (Rustomjee 2008). While TMC207 demonstrated a delayed onset in bactericidal activity, it was well tolerated, and no serious adverse events occurred (Rustomjee 2008). For multidrug-resistant tuberculosis, the addition of TMC207 to standard therapy reduced the time to conversion to a negative sputum culture (Diancon 2009). TMC207 displays no cross-resistance to other antituberculosis drugs, as it has a novel site of action. It also has the potential to shorten the duration of antituberculosis treatment. It is, however, metabolized by CYP, which will necessitate dose-adjustment if administered simultaneously with rifampin (Global Alliance 2007, Andries 2005, Cole 2005). This will be very difficult to manage due to the long half-life of TMC207.

Gatifloxacin

Gatifloxacin is a fluoroquinolone that inhibits DNA gyrase, thus inhibiting *M.tb* DNA replication and transcription. The OFLOTUB Consortium, the European Commission, WHO TDR and Lupin Ltd are among its sponsors and co-ordinators. Gatifloxacin is currently undergoing phase 3 clinical trials (Ginsberg 2007). Gatifloxacin is a front-runner as the first antituberculosis agent to reduce pulmonary antituberculosis treatment to 4 months' duration. There are weak data to support its efficacy against MDR-TB (Global Alliance 2007, Hu 2003). However, there have been concerns about dysglycaemia with gatifloxacin (Park-Wyllie 2006).

Moxifloxacin

Moxifloxacin is also a fluoroquinolone and has a similar mechanism of action to gatifloxacin. It is undergoing phase 2 and 3 clinical trials by CDC TBTC, Johns Hopkins University and UK MRC. The TB Alliance and Bayer are jointly pursuing clinical development for antituberculosis drugs. Moxifloxacin kills rifampin-tolerant persisters *in vitro*, and it may help treat MDR-TB if co-administered with ethionamide. Data from the mouse model support the possibility that moxifloxacin, when substituted for isoniazid or ethambutol in standard, first-line antituberculosis treatment, will increase the 2-month sputum conversion rate and potentially shorten overall time to stable cure. It may thus shorten duration of antituberculosis treatment (Global Alliance 2007, Nuermberger 2005, Hu 2003, Burman 2006). In a phase 2 trial, moxifloxacin was associated with a bi-exponential fall in colony counts during the early phase of antituberculosis treatment; this was superior to gatifloxacin, ofloxacin and ethambutol (Rustomjee 2008). Recently, in a phase 3 trial, moxifloxacin showed significant increases in week-8 culture negativity, compared to ethambutol. When substituted for isoniazid, moxifloxacin had a small but statistically non-significant increase in week-8 culture negativity (Dorman 2009).

SQ-109

SQ-109 is an ethylenediamine and is derived from ethambutol. It is postulated to inhibit cell wall biosynthesis; its intracellular targets have yet to be elucidated. Sequella Inc. (in collaboration with the National Institutes for Health) is currently conducting phase 1B clinical trials (Ginsberg 2007). Current data suggest that SQ-109 is effective against drug-resistant strains (including ethambutol-resistant strains). SQ-109 appears to be synergistic with isoniazid and rifampin (Global Alliance 2007, Chen 2006).

Pyrrole LL3858

Limited data are available regarding pyrrole LL3858. It is currently undergoing phase 1 clinical trials by Lupin Limited (India) (Global Alliance 2007). Available data suggest that LL3858 has potency against standard and drug-sensitive *M.tb* strains in vitro (Global Alliance 2007, Ragno 2000).

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7.2.2 Antiretroviral treatment in development

Second generation agents

Etravirine (TMC125)

Etravirine, also known as TMC-125, is a next-generation NNRTI. It is a highly flexible, diaryl-pyrimidine (DAPY) compound that enables favourable binding interactions with mutant HIV-1 strains as well as with wild-type virus (Boone 2006). Developed by Tibotec (Johnson & Johnson), Etravirine has completed phase 3 clinical trials and is licenced by the FDA (Towner 2010). When combined with a background regimen, such as darunavir/ritonavir and/or raltegravir, etravirine provided an effective treatment option in treatment-experienced patients with HIV-1 (Boone 2006).

Darunavir (TMC 114)

Darunavir is a second generation PI that is administered with low-dose ritonavir. It is relatively resistant to mutations that confer PI resistance (de Bethune 2006). In phase 3 trials of ART-experienced patients, darunavir was an effective treatment option (Towner 2010). It is now also licenced by the FDA.

New class agents

CCR5 inhibitors

Maraviroc

Maraviroc, also known as UK-427 and 857, is a CCR5 antagonist. It blocks the CCR5 co-receptor on CD4+ cells, thus preventing CCR5-tropic HIV-1 from entering CD4+ cells.

Manufactured by Pfizer, it was approved for use by the FDA on 24 April 2007. Furthermore, it was granted accelerated approval on 6 August 2007 for combination ART of adults infected only with detectable CCR5-tropic HIV-1, who have evidence of viral replication and have HIV-1 strains resistant to multiple anti-retroviral agents (The Pinksheet 2007). Maraviroc is a substrate for CYP3A4, so it may interact with rifampin, NNRTIs and PIs; levels of maraviroc are increased in patients taking atazanavir, ritonavir-boosted lopinavir (Kaletra) and ritonavir-boosted saquinavir (Invirase) (Reeves 2005).

Vicriviroc (SCH-D)

Vicriviroc is also a CCR5 antagonist and has a similar mechanism of action to maraviroc. It is being developed by Schering-Plough. In phase 2 clinical trials conducted in treatment-experienced patients, vicriviroc administered with a protease inhibitor and ritonavir-containing regimen showed potent antiretroviral and immunologic activity sustained over 48 weeks (Suleiman 2010). Vicriviroc was discontinued in treatment-naïve patients because of poor virological outcomes. In one study, five patients in the vicriviroc arm developed malignancies, but the Drug and Safety Monitoring Board could not determine a causal link so the study proceeded (Reeves 2005).

Integrase inhibitors: raltegravir and elvitegravir

Raltegravir, also known as MK-0518, is an integrase inhibitor and inhibits HIV-1 DNA integration into host DNA. Developed by Merck, it underwent phase 3 clinical trials and showed comparable efficacy to enfuvirtide (de Castro 2009). Raltegravir's safety profile is comparable to placebo, and it has demonstrated virological benefit in salvage ART (Cooper 2007) and first-line ART. It is metabolised by glucuronidation (UGT1A1) and has no significant effect on CYP3A4 (Reeves 2005, Cooper 2007, Pommier 2005). Elvitegravir, known as Gilead-9137, is also an integrase inhibitor and has a similar mechanism of action to raltegravir. Elvitegravir demonstrates efficacy against multi-drug-resistant HIV-1 and has a side effect profile similar to placebo. It is metabolised by glucuronidation and CYP, so it has important drug interactions. It is also an inducer of CYP3A4. In clinical studies, it is used with ritonavir boosting to increase its half-life from three to nine hours, increase its AUC and prevent auto-induction of its metabolism (Reeves 2005, Pommier 2005, Mathias 2006). The FDA has also licensed raltegravir and elvitegravir.

Maturation inhibitor: PA-457

PA-457, also known as DSB, is a maturation inhibitor and blocks HIV-1 maturation by disrupting cleavage of the capsid precursor. Panacos Pharmaceuticals has recently completed phase 2 clinical trials (Smith 2007). Single oral doses of PA-457 were well tolerated, and there was a dose-dependent reduction in viral load. Encouragingly, no PA-457 resistance mutations were detected during the course of the study (Reeves 2005, Li 2006).

7.2.3 *Future areas of research*

Insight into how *M. tb* persists may illuminate novel ways to reduce the prolonged duration of antituberculosis treatment and the emergence of drug resistance. In particular, a recent advance has occurred in understanding *M.tb*'s adaptation to hypoxia, which is theoretically important in latent lesions (Voskuil 2003). It is also increasingly clear that metabolic adaptation to persistence involves a switch to fatty acid metabolism as a source of carbon; deletion of both genes encoding isocitrate lyases 1 and 2 impairs the persistence of *M.tb* (Munoz-Elias 2005). The absence of these gene products in humans may facilitate the development of glyoxylate cycle inhibitors as new drugs for the treatment of tuberculosis (Munoz-Elias 2005).

In summary, many novel and efficacious antituberculosis and antiretroviral drugs are swelling the drug development pipeline. Reassuringly, these drugs promise to alleviate the complications and challenges inherent in the current drug regimens.

7.3 Concluding remarks

In this thesis, I related my experiences of clinical deterioration during antituberculosis treatment. Two important issues emerged: the association of clinical deterioration with HIV-1, and the difficulties encountered in the diagnosis and management of these profoundly ill patients. Reducing the extraordinary burden of clinical deterioration during antituberculosis treatment is clearly an urgent priority for South Africa.

Numerous efforts have already been made, which merit acknowledgement. South African-based research centres are empowering and training local and international clinician scientists. Strong local and international funding supports MDR-TB and TB-IRIS research. Recently, the South African government announced it would consider increasing the entry CD4+ count to $<350\text{cells}/\mu\text{L}$ in patients with tuberculosis and pregnancy. Furthermore, philanthropic organisations, pharmaceutical companies and the TB alliance have re-invigorated the tuberculosis and HIV drug development pipeline. Novel drugs promise to improve combined antituberculosis and antiretroviral treatment with the mitigation of shared drug toxicities, drug resistance and the long duration of antituberculosis treatment. It is my hope that these research ventures will impact those most affected by HIV-1 and tuberculosis. All current efforts will be in vain without improving the infrastructure of DOTS and ART programmes, as well as garnering social and political commitment.

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Appendices

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Publications

Arising from the thesis

1. **Pepper DJ**, Meintjes G, McIlleron H, Wilkinson RJ. Combined therapy for tuberculosis and HIV-1: the challenge for drug discovery. *Drug Discovery Today*. **2007**; 12 (21-22):980-989
2. Oni T, **Pepper DJ**, Wilkinson RJ. HIV-TB Drug Interactions chapter in *AIDS and Tuberculosis: A Deadly Liaison*. **2009**; Infection Biology Handbook Series. Wiley Blackwell. pg. 123-153.
3. **Pepper DJ**, Rebe K, Morroni C, Wilkinson RJ, Meintjes G. Clinical deterioration during antitubercular treatment at a district hospital in South Africa: the importance of drug resistance and AIDS defining illnesses. *PloSONE* **2009**; 4(2):e4520
4. **Pepper DJ**, Marais S, Maartens G, Morroni C, Rebe K, Rangaka MX, Oni T, Wilkinson RJ, Meintjes G. Neurological Manifestations of Paradoxical Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome: A Case Series. *Clinical Infectious Diseases*. **2009**; 48 (11):e96-107
5. **Pepper DJ**, Marais S, Wilkinson RJ, Bhaijee F, Maartens G, McIlleron H, De Azevedo V, Cox H, McDermid C, Sokhela S, Patel J, Meintjes G. Clinical Deterioration during Antituberculosis Treatment in Africa: Incidence, Causes and Risk Factors. *BMC Infectious Diseases*. **2010**; 10:83

6. Marais S, Scholtz P, **Pepper DJ**, Meintjes G, Wilkinson RJ, Candy S. Neuroradiological features of the tuberculosis-associated immune reconstitution inflammatory syndrome. *International Journal of Tuberculosis and Lung Disease*. **2010**;14(2):188-96.
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8. Bhaijee F, Wainwright H, Meintjes G, Wilkinson RJ, Todd G, de Vries E, **Pepper DJ**. Warfarin Induced Skin Necrosis in HIV-1 Infected Patients with Tuberculosis and Venous Thrombosis: a case series. **2010**; *South African Medical Journal*. [in press]

Relevant to the thesis

9. Meintjes G, Lawn SD, Scano F, Maartens G, French MA, Worodria W, Elliott JH, Murdoch D, Wilkinson RJ, Seyler C, John L, van der Loeff MS, Reiss P, Lynen L, Janoff EN, Gilks C, Colebunders R; International Network for the Study of HIV-associated IRIS. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. *Lancet Infect Dis*. 2008 Aug;8(8):516-23.
10. Meintjes G, Rangaka MX, Maartens G, Rebe K, Morroni C, **Pepper DJ**, Wilkinson KA, Wilkinson RJ. Novel relationship between tuberculosis immune reconstitution inflammatory syndrome and antitubercular drug resistance. *Clin Infect Dis*. 2009 Mar 1;48(5):667-76.



Combined therapy for tuberculosis and HIV-1: the challenge for drug discovery

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Combining drug therapies for dual infection by *Mycobacterium tuberculosis* and HIV-1 is made complex by high pill burdens, shared drug toxicities, drug–drug and drug–disease interactions, immune reconstitution inflammatory syndrome, co-morbid diseases and drug resistance in both bacillus and virus. Recently, novel anti-tubercular and anti-retroviral drugs have bolstered the tuberculosis–HIV drug pipelines and may help ameliorate these difficulties. This review article discusses the reasons for current problems of therapy for dual infection. It also identifies promising agents, which may significantly improve co-therapy and thus diminish the great morbidity and mortality of these two pandemics.

The dual pandemics of tuberculosis (TB) and human immunodeficiency virus-1 (HIV) cause considerable morbidity and mortality. One-third of the world's population is infected with *Mycobacterium tuberculosis* (TB), and almost nine million new cases of TB and approximately two million TB deaths occur annually [1,2]. Worldwide, almost 40 million people were infected with HIV and 2.9 million people died with AIDS in 2006 [3]. In 2000, almost 11 million people were co-infected with HIV and TB [1], the majority of who were in the developing world [4]. These two pandemics fuel each other. TB hastens HIV progression to AIDS by accelerating viral replication [5], whilst HIV increases the risk of TB disease (up to 30% annual risk of TB disease in profoundly immune-suppressed patients in endemic areas) [6]. TB–HIV drug therapy is complicated by high pill burdens, shared drug toxicities, drug–drug and drug–disease interactions and the immune reconstitution inflammatory syndrome. Co-morbid diseases, drug resistance and the treatment of latent TB infection provide additional challenges. This review article describes current therapy for TB and HIV, discusses the problems with current

TB–HIV therapy, reviews new drugs on the horizon and proposes areas for future research.

Current therapy for tuberculosis and HIV

TB therapy is a multi-drug regimen given over a long period of time. Single agent TB therapy rapidly gives rise to drug-resistant organisms [7]. Multi-drug treatment needs to be prolonged; possible explanations include: *M. tuberculosis* divides slowly, it is metabolically capable of becoming drug insensitive and/or bacilli may become sequestered [8]. The advent of rifampicin and pyrazinamide allowed highly effective 'short course' TB Regimes—usually a two-month intensive phase with rifampicin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E), followed by a four-month continuation phase with RH (2RHZE/4RH) [9]. Multi-drug-resistant (MDR)-TB, defined as TB resistant to RH, is treated with less effective agents for up to 18 months after sputum conversion. Treatment is often individualised and regimes may be as long as 24 months. Patient adherence is a major problem with such prolonged treatment regimens. There is an urgent need for shorter, effective TB drug regimens.

Highly active anti-retroviral therapy (HAART or ART) for HIV is a life-long multi-drug regimen. In patients infected with HIV,

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millions of virions are produced daily, and the reverse transcriptase target mutates rapidly. Hence, initial therapies with single or dual nucleoside reverse transcriptase inhibitors (NRTI) such as zidovudine (AZT) and didanosine (ddI) were only partially effective and rapidly led to viral drug resistance [10]. Effective therapy only became possible when non-nucleoside reverse transcriptase (NNRTI) and viral protease inhibitor (PI) drugs were developed. Combinations of these three drug classes lead to prolonged suppression of HIV replication and ultimately to a degree of immune recovery. Adherence to ART is crucial to successful viral suppression, which is very closely related to immune restoration and survival [11].

Difficulties associated with combining current TB–HIV therapies

Shared drug toxicities

Concomitant therapy for HIV and TB is associated with increased risks of adverse drug effects such as nausea, gastrointestinal tract disturbance, peripheral neuropathy, cutaneous reactions, renal toxicity and potentially fatal liver toxicity [12]. Certain drug combinations are contraindicated (Table 1). These toxicities may necessitate therapy discontinuation, which exacerbates immune suppression and predisposes to other opportunistic infections. Shared drug toxicities also compromise adherence to the treatment regimen leading to suboptimal TB and HIV treatment and increasing the possibility of drug resistance.

Drug interactions

Clinically significant pharmacokinetic drug interactions are common in TB–HIV therapy. Many TB–HIV drugs are substrates of metabolising enzymes and drug transporters that are induced or inhibited by key components of anti-TB and ART regimens.

Rifamycins form the backbone of regimens for drug-susceptible TB because they are highly effective, reduce the duration of therapy and are less likely than other TB drugs to select out resistant strains in multi-drug regimens [13]. However, through its activation of a master transcriptional regulator, the pregnane X receptor (PXR), rifampicin induces the expression of a broad array of enzymes and drug-transporting molecules including cytochrome P450 (CYP) 3A and CYP2C isoenzymes, CYP2D6, CYP2B6 and *p*-glycoprotein, amongst others [14]. Thus, repeated doses of rifampicin result in clinically important reductions in the levels of many drugs, amongst them PIs, NNRTIs, trimethoprim and sulfamethoxazole (the last two co-formulated in co-trimoxazole widely used for prophylaxis in patients with advanced HIV-1 infection) [15,16]. The co-localization of *p*-glycoprotein and CYP enzymes in enterocytes, hepatocytes and renal tubular cells may enhance the effects of rifampicin on common substrates, such as PIs, causing more extensive pre-systemic metabolism and accelerated drug elimination. The clinical consequences of rifampicin-related decreases in serum concentrations of the anti-retroviral drugs have not been fully studied, but they potentially lead to loss of anti-viral efficacy and stepwise accumulation of resistance mutations [17–20]. Whilst NNRTIs, besides delavirdine, can be given with rifampicin [21], dose increase may be necessary. All PIs, except ritonavir-boosted PIs, are contraindicated with rifampicin [22]. Certain ritonavir-boosted PIs can be used with rifampicin, but increased doses of ritonavir or a higher dose of the companion PI are required.

The rifamycin rifapentine has a longer half-life suitable to intermittent administration that might simplify therapy. Whilst rifapentine is also a marginally less potent inducer of CYP than rifampicin it has not been widely introduced as it is associated with increased rates of TB drug resistance when used once weekly during the continuation phase of TB therapy [23]. Intermittent rifampicin therapy in the continuation phase is also associated with rifampicin resistance in patients with advanced HIV infection [24]. Rifabutin, the rifamycin causing least induction of CYP enzymes, is safe to use with most NNRTIs and PIs, except delavirdine and saquinavir. However, it is also a substrate for CYP3A4 [25]. Thus, its serum concentration and toxicity are increased when co-administered with PIs and decreased when co-administered with efavirenz and RFB dose adjustments are required in these settings [15]. Rifabutin and the anti-retroviral nevirapine can be given together at standard doses, but delavirdine is contraindicated with rifabutin [15]. Also, the cost of rifabutin precludes its use in the developing world.

Although they are of lesser importance, other pharmacokinetic interactions may confound combined treatments. Isoniazid is an inhibitor of CYP2C19 and CYP3A, PIs inhibit CYP3A, CYP2D6 and *p*-glycoprotein, and efavirenz and nevirapine induce the expression of CYP3A4 and CYP2B6. Other commonly co-administered drugs also cause important changes in anti-retroviral concentrations through induction or inhibition of CYP isoforms (e.g. anti-convulsants like carbamazepine and phenytoin may decrease, and azole anti-fungals, macrolide antibiotics and H2 antagonists may substantially increase PI or NNRTI concentrations).

Pharmacodynamic interactions further complicate combined treatment of TB and HIV. Unanticipated hepatotoxicity has been reported in healthy volunteers receiving adjusted dose PIs in combination with rifampicin [26,27]. Interestingly, repeated doses of RIF before the introduction of the PIs appear to be associated with a higher risk of hepatotoxicity than the introduction of rifampicin after establishing regular doses of the PIs. Although not adequately studied, such high rates of hepatotoxicity have not been reported in patients receiving increased doses of PIs with rifampicin-based TB regimens, indicating that disease-related modulation of hepatotoxicity can occur.

The effect of HIV infection on the concentrations of orally administered first-line anti-tubercular drugs is also a concern. Whilst currently available studies are not entirely consistent, it appears that HIV-infected patients achieve not only somewhat lower concentrations of rifampicin and ethambutol in particular but also isoniazid and pyrazinamide [28–31]. Patients with more advanced HIV disease and those with diarrhoea appear to be at most risk. Although these concentration reductions do not appear to have a marked effect on treatment outcomes their importance has not been fully evaluated.

High pill burden

Combined therapies for TB and HIV together with the recommended co-trimoxazole prophylaxis lead to a very high pill burden—average of 15 pills daily in South Africa for 6–22 months (fixed dose combinations (FDC) for TB medication but not ART). Furthermore, in high burden countries standard treatment approaches are adopted and FDCs are often used to reduce the pill burden and to simplify drug supply, prescribing and admin-

TABLE 1

Shared drug toxicities in HIV/TB patients

Side-effect	Anti-retrovirals	First-line TB drugs	MDR-TB drugs	Other drugs commonly used in HIV	Other HIV disease
Peripheral neuropathy	d4T, ddl	H, E (rare)	Cy, Te, FQ, Et, Ka, Am, Lin		HIV itself
Hearing loss		S	Ka, Am, Cpr, Clr		
Vertigo		S	Ka, Am, Clr		
Optic neuritis		E, H (rare)	Et (rare), PAS (rare), Lin		
Seizures		H	Cy, Te, Of, Ci [and other FQ – rare]		
Psychosis	EFV	H	Cy, Te, Of, Ci, [other FQ] Et	TMP-SMX, steroids	HIV itself
Depression	EFV		Cy, Te, Of, Ci, Et	TMP-SMX	HIV itself
Nausea & vomiting	AZT, ddl, PI: IDV, amprenavir (other PIs)	Z, Rfm, H, E	Et, Of, Ci, [other FQ], PAS, Clf, Lin	TMP-SMX, Amphotericin B	OI, IRIS
Gastritis		E, Z	Et		
Transaminitis	NVP, PI, EFZ	Z, R, H	Of, Ci [other FQ], Et, Cy, Te, PAS, Clr	TMP-SMX, Azoles	OI
Hepatic steatosis	d4T				OI, IRIS
Cholestasis		R	Clr	TMP-SMX	AIDS cholangiopathy
Nephrotoxicity	TDF (including Fanconi syndrome)	S, R (interstitial nephritis & GN), Z+H also rarely cause interstitial nephritis	Ka, Am, Cpr, FQ – rarely cause IN, PAS causes crystalluria	Amphotericin B, TMP-SMX (interstitial nephritis)	HIVAN
Hypokalaemia	TDF		Ka, Am, Cpr	Amphotericin B	
Renal calculi	IDV			TMP-SMX	
Arthralgias and gout		E, Z, H, R	Of, Ci, [other FQ, PAS]	Thiazide (gout)	HIV itself
SJS/TEN	NNRTIs	R, H (both rarely)	Thiacetazone, Clr, PAS, FQ	TMP-SMX	
Skin rash	NNRTIs, ABC, PI	Z, R, H, S, E	FQ, Clf, PAS, Clr, Cpr, Et, Cy, Te	TMP-SMX	Folliculitis & asteatosis
Hypersensitivity	ABC	R, S		TMP-SMX	
Leucopenia, anaemia	AZT, 3TC	R, H, Z (sideroblastic anaemia), RHZE rarely cause thrombocytopenia	FQ, strep, Cy (megaloblastic), PAS, Lin, Cpr, Clr (last 4 also thrombocytopenia)	Ganciclovir, TMP-SMX, Amphotericin B	HIV itself
Lactic acidosis	d4T, ddl, AZT		Lin		
Pancreatitis	ddl, d4T	H	Lin, Clr	TMP-SMX	
Insulin resistance	PI esp IDV			steroids	
Hyperlipidaemia	PI (except ATV), d4T			steroids	
Lipoatrophy	d4T				
Lipodystrophy	PIs				
Hep B flare if drug discontinued	TDF, FTC, 3TC				
Teratogenic	EFV	S	Et	steroids	
Osteoporosis	TDF			steroids	

Antiretrovirals D4T = stavudine, ddl = didanosine, Efv = efavirenz, AZT = zidovudine, IDV = indinavir, PI = Protease inhibitor, NVP = nevirapine, TDF = tenofovir, NNRTI = Efv + NVP, ABC = abacavir APV. **First-line TB drugs** Rfm = rifamycins, R = rifampicin, H = isoniazid, E = ethambutol, Z = pyrazinamide, S = streptomycin. **MDR-TB drugs** Cy = cycloserine, Te = Terizidone, Of = Ofloxacin, Ci = Ciprofloxacin, Et = Ethionamide[+prothionamide], Ka = Kanamycin, Am = Amikacin, FQ = fluoroquinolones, Lin = linezolid, Cpr = capreomycin, Clr = clarithromycin, Clf = clofazimine, PAS = p-amino-salicylate. TMP-SMX = trimethoprim sulfamethoxazole, IRIS = immune reconstitution inflammatory syndrome, OI = opportunistic infections. Drugs in boldface should not be used simultaneously because they potentiate the drug side-effect or are associated with significantly increased morbidity or mortality.

istration. Pharmacokinetic interactions necessitating dose adjustment of individual drug components complicate treatment delivery in the setting of programmes reliant on standardised approaches using FDCs. Thus, optimisation of therapy inevitably leads to a greater pill burden.

Immune reconstitution inflammatory syndrome

Immune reconstitution inflammatory syndrome (IRIS), which is due to dysregulated immune recovery [32], occurs in severely immune-suppressed HIV patients typically one to four weeks after ART initiation [33,34]. In tuberculosis-related IRIS an exuberant inflammatory reaction is directed towards mycobacterial antigens [35], resulting in worsening pulmonary infiltrates, pleural effusions, lymphadenitis and potentially fatal neurological tuberculomata [33,34]. Risk factors for TB-IRIS include low baseline CD4 count, high baseline viral load, short duration between TB and ART initiation and disseminated tuberculosis [33,36]. Determining the optimal time to initiate ART in severely immune-suppressed TB patients is difficult [12,25]. Whilst early initiation of ART may increase the risk of TB-IRIS, non-adherence, drug toxicities and drug interactions, the risk of death, other opportunistic infections (OIs) and malignancies may be greater if ART initiation is delayed [37]. Corticosteroid treatment of TB-IRIS is sometimes recommended, but such immunosuppressive therapy may be associated with reactivation of occult OIs and malignancies, such as CMV and Kaposi's sarcoma. A randomised trial of corticosteroids against placebo for TB-IRIS would help resolve this issue.

Co-morbid diseases

HIV-induced immunosuppression, predisposing to OIs and malignancies, coupled with TB-HIV drug side effects and TB-IRIS can give rise to multiple pathologies in certain organs, especially the liver (Table 2). When faced with significant biochemical or clinical evidence of hepatitis it is a dilemma whether to withdraw all drugs or the most likely offending candidates. Evidence certainly suggests withdrawal of rifampicin, isoniazid, pyrazinamide and NNRTI medications, the latter under the cover of a two NRTI 'tail'

to reduce the risk of NNRTI resistance. Substituting 'liver friendly' alternatives such as streptomycin and quinolones to continue the treatment of TB is of uncertain efficacy. Investigating these differential diagnoses without the use of invasive techniques such as endoscopic retrograde cholangio-pancreatography or biopsy poses major challenges to clinicians in resource-limited settings. A further problem on resolution of the hepatitis is when and how drugs should be re-introduced. No attempt to re-introduce nevirapine therapy should be made after significant drug-induced toxicity, but it is common experience that anti-TB drugs may all be re-introduced. This is usually done sequentially with monitoring of clinical signs and hepatic enzymes but is a prolonged empirical exercise that maintains the patient in expensive hospital care and at risk of nosocomial infection. Well-conducted clinico-pathological studies of adequate numbers of patients are required to address these issues and inform practice.

Drug-resistant HIV

The prevalence of multi-drug-resistant HIV is increasing [38]. This may be accounted for, partly, by the transmission of HIV-1 from drug-experienced to uninfected individuals [39]. Whilst drug resistance (DR) is not associated with increased virulence [40], DR is the leading cause of treatment failure amongst patients infected with HIV [41]. High adherence (>95%) to ART reduces viral replication, which limits the emergence of drug-resistance mutations [42]. Intermediate ART adherence (70–90%) and ART monotherapy or dual therapy increase the incidence of DR [11]. Recent data suggest that moderate adherence to NNRTIs leads to sustained viral suppression [11]. Certain reverse transcriptase mutations conferring resistance to NRTI (K65R, M184V) are associated with reduced viral fitness [43], whilst mutations conferring NNRTI resistance are usually neutral. Interestingly, NNRTI hypersusceptibility is more common amongst viruses from NRTI experienced/NNRTI-naïve patients compared with viruses from NRTI/NNRTI-naïve patients, suggesting that mutations in NRTI-resistant viruses confer structural conformational advantages for NNRTIs at the NNRTI-binding site [44]. Reduced viral fitness of DR HIV-1

TABLE 2

Causes of liver disease in TB-HIV patients

	Drugs	Infections	Malignancies
Transaminitis (ALT >3 times normal)	TB drugs: Rif, INH, Z ART: NNRTI, PI Azoles TMP-SMX	Viral Hepatitis A, B, C CMV EBV	
Canalicular pattern (ALP >3 times normal)			
Infiltration	NRTI (steatosis)	Granulomatous hepatitis TB/Mycobacterium avium-intracellulare/TB-IRIS Fungal CMV	Kaposi's sarcoma Lymphoma
Cholangiopathy (USS liver/ERCP)	Rif TMP-SMX	CMV Salmonella, Campylobacter, Isospora belli, Microsporidium, Cryptosporidium	
Enzyme induction	Macrolides		

- Certain protease inhibitors (atazanavir and indinavir) can cause unconjugated hyperbilirubinaemia
- Sepsis causes transaminitis with conjugated hyperbilirubinaemia
- If non-specific hepatitis (ALT <3 times normal) then consider malnutrition, NRTIs, ethanol, herbal medication and viral hepatitis serology

strains allows their growth to be exceeded by wild-type HIV strains [45]. The detection of DR HIV-1 in ART-naïve patients may consequently be difficult, as standard population-based genotyping methods only detect viral populations that are greater than 20% of the total HIV-1 population [45]; drug pressure allows the subsequent detection of DR strains [38]. In addition, DR is increased when a failing ART regimen is maintained (even though stable levels of CD4 and VL are preserved over time) and may limit future treatment options [46,47]. New HIV drugs are thus required to overcome cross-resistance and offer alternative therapy in treatment failure.

Drug-resistant tuberculosis

The continued emergence of drug-resistant TB [48] may be due to several factors: poor directly observed treatment short course (DOTS) implementation, the possible greater biological propensity of some strains of TB to become drug resistant (e.g. W-Beijing [49]) and HIV co-infection that is likely to be associated with greater bacterial burdens. W-Beijing strains of TB have been associated with HIV and a greater propensity to become drug resistant. Biologically these strains are postulated to interact in an immune subverting manner because they produce an immunosuppressive phenolic glycolipid [50]. National DOTS programmes require substantial infrastructure and political commitment, which are often suboptimal in the developing world. TB-HIV co-infection is also associated with significantly reduced RIF drug levels, which may allow the selection of drug-resistant TB bacilli [28,30]. Patients receiving MDR-TB treatment may also be at risk of extensively drug-resistant (XDR) TB (defined as MDR plus resistant to at least a quinolone and a second line injectable drug [51,52]) as current MDR-TB therapy is prolonged, poorly tolerated and less effective than first-line TB therapy. Worryingly, the nosocomial acquisition of XDR-TB has recently been documented [53]. Rapid diagnostic methods to ascertain drug resistance and correct infrastructure augmented by a substantial financial commitment by both first and third world countries will be required to combat the expanding TB pandemic and prevent the spread of drug-resistant TB [13].

Treatment of HIV-1-associated latent tuberculosis infection

ART reduces the incidence of TB, though it remains to be seen whether this benefit may be offset by increased survival and an overall increase in lifetime risk. Treatment of latent TB infection (LTBI) also reduces the risk of subsequent TB disease even in HIV-infected people, though the duration of protection is relatively short [54]. It therefore appears logical to potentially combine therapies to prevent TB. However, the risk benefit of preventive TB therapy in these circumstances is unknown: There are significant overlapping hepatic and neurological toxicities as outlined above. In addition, the most effective preventive therapy is isoniazid monotherapy, but this may be associated with an increased risk of isoniazid resistance if inadvertently given to patients with active disease [55]. Therefore, whilst the prescribing of either isoniazid or ART has significant benefits in HIV-infected persons, a randomised controlled trial is still needed to determine the efficacy and risks of combination isoniazid/ART therapy. The combination regimen of rifampicin and pyrazinamide for two months was associated with liver toxicity especially in HIV-uninfected persons and is no longer recommended [56]. There is very

little evidence to guide prescription of preventive therapies in persons exposed to MDR-TB [57].

Drugs on the horizon

In order to meet the challenges of TB-HIV co-infection, new TB and HIV drugs are urgently needed. These new TB drugs should shorten the duration of TB therapy or significantly reduce the number of doses under DOTS, have efficacy against both drug-susceptible and MDR-TB, be effective in treating LTBI, have a new site of drug action thus limiting cross-resistance and not be metabolised by CYP or induce or inhibit CYP, thus limiting shared toxicities between HIV and TB therapy. Since 2000, several novel TB and HIV drug candidates have been identified (Tables 3 and 4 [58,59]).

TB drugs in development

PA-824

PA-824 is a nitroimidazo-oxazine. It requires activation by *M. tuberculosis* F420 factor and inhibits synthesis of cell wall lipids as well as protein synthesis. The TB alliance is currently conducting phase 1 clinical trials of PA-824 [60]. Preliminary studies suggest that PA-824 will be active against MDR-TB and has no cross-resistance with other anti-tubercular drugs. Importantly, it is not metabolised by CYP and does not induce or inhibit CYP. It had similar bacteriostatic efficacy to rifampicin and was more efficient than isoniazid or moxifloxacin but less efficient than rifampicin

TABLE 3

TB-HIV drugs in development

TB	HIV
<i>Novel chemical entities</i>	Attachment inhibitors
ATP synthase inhibitor:	Dextran sulfate
R 207910/diarylquinoline TMC 207	Heparin
FAS20013	Cyanovirin-N
Cell wall inhibitors:	Cyclotriazadisulfonamide analogues
Nitroimidazo-oxazine PA-824	PRO 2000
Nitroimidazo-oxazole OPC-67683	TNX 355
Dipiperidine SQ-609	PRO 542
Translocase I inhibitor	BMS 806
InhA inhibitors	Co-receptor binding inhibitors
Isocitrate lyase inhibitors	CCR5: SCH-D
Protein synthesis inhibitor	Maraviroc
Pyrrole LL-3858	Aplaviroc
Other: Pleuromutilins	TAK 779
	Ancriviroc
	CXCR4: AMD 070
<i>Based on existing chemical entities</i>	Plerixafor
Fluoroquinolones	Fusion inhibitors
Moxifloxacin	Tifuvirtide
Gatifloxacin	
New quinolones	
Non-fluorinated quinolones	Non-nucleoside reverse transcriptase inhibitors
Ethambutol derivative:	Etravirine
SQ-109	Integrase inhibitors
Macrolides	L 731988
Thiolactomycin analogues	L 870810
Nitrofuranylaminines	L 870812
Rifamycin derivatives:	Maturation inhibitors
Rifalazil	PA 457
Oxazolidinones:	Protease inhibitors
Linezolid	Darunavir

and isoniazid in continuation phase therapy in the mouse model [58,61,62].

OPC-67683

OPC-67683 is a nitroimidazo-oxazole that is similar in structure to PA-824. It inhibits cell wall biosynthesis. Otsuka Pharmaceuticals (Japan) are currently conducting phase 2 clinical trials [60]. Pre-clinical studies in rodents and dogs suggest that OPC-67683 could be used in HIV/AIDS as it has no effect on CYP. It may have treatment-shortening potential as it synergises *in vitro* with rifampicin and pyrazinamide. OPC-67683 is effective against MDR-TB *in vitro* and displayed no cross-resistance to first line TB therapy. It also has potential to treat LTBI [58,63].

R207910

R207910 is a diarylquinoline and is also known as Compound J and TMC207. It inhibits ATP synthase (AtpE) leading to ATP depletion and pH imbalance [64,65]. It was initially identified by Johnson & Johnson and subsequently developed by the subsidiary Tibotec Pharmaceuticals Limited, where it is undergoing phase 2a clinical trials in both drug sensitive and resistant disease [60]. Murine studies suggest that R207910 has a good safety and tolerability profile and potent early bactericidal activity, matching isoniazid. It had a synergistic effect with pyrazinamide for MDR-TB; R207910/H/Z or R207910/R/Z combinations were more effective than amikacin/Z/moxifloxacin/ethionamide regimens. R207910 displayed no cross-resistance to other TB drugs as it has a novel site of action. It also has the potential to shorten duration of TB therapy. Its drawback is its metabolism by CYP, which will necessitate dose-adjustment if administered simultaneously with rifampicin [58,64,65].

Gatifloxacin

Gatifloxacin is a fluoroquinolone that inhibits DNA gyrase, thus inhibiting TB DNA replication and transcription. Its sponsors and co-ordinators include the OFLOTUB Consortium, the European Commission, WHO TDR and Lupin Ltd. Gatifloxacin is currently undergoing phase 3 clinical trials [60]. Gatifloxacin holds the potential to be the first TB agent to reduce pulmonary TB therapy to four-month duration. There are weak data to support its efficacy against MDR-TB [58,66]. However, there have been concerns about dysglycaemia with gatifloxacin [67].

Moxifloxacin

Moxifloxacin is also a fluoroquinolone and has a similar mechanism of action to gatifloxacin. It is undergoing phase 2 and 3 clinical trials by CDC TBTC, Johns Hopkins University and UK MRC. The TB alliance and Bayer are jointly pursuing clinical development for tuberculosis. Moxifloxacin kills rifampicin-tolerant persisters *in vitro*, and it may help treat MDR-TB if co-administered with ethionamide. Data from the mouse model support the possibility that moxifloxacin when substituted for isoniazid or ethambutol in standard, first-line TB treatment will increase the two-month sputum conversion rate and potentially shorten overall time to stable cure. It may thus shorten duration of TB therapy [58,62,66,68].

SQ-109

SQ-109 is an ethylenediamine and is derived from ethambutol. It is postulated to inhibit cell wall biosynthesis and has intracellular

targets, which have not yet been elucidated. Sequella Inc. (in collaboration with the National Institutes for Health) is currently conducting phase 1 clinical trials [60]. Current data suggest that SQ-109 is effective against drug-resistant strains (including ethambutol-resistant strains). SQ-109 appears to be synergistic with isoniazid and rifampicin [58,69].

Pyrrole LL3858

Limited data are available regarding pyrrole LL3858. It is currently undergoing phase 1 clinical trials by Lupin Limited (India) [58]. Available data suggest that LL3858 has potency against standard and drug-sensitive TB strains *in vitro* [58,70].

HIV drugs in development

Second generation agents

Etravirine (TMC125)

Etravirine, also known as TMC-125, is a next-generation NNRTI. It is a highly flexible, di-aryl-pyrimidine (DAPY) compound that enables favourable binding interactions with mutant HIV strains as well as wild-type virus [71]. It is being developed by Tibotec (Johnson & Johnson) and is currently in phase 3 clinical trials and may become the first NNRTI suitable for use in NNRTI-experienced patients [71,72].

Darunavir (TMC 114)

Darunavir is a second generation PI that is administered with low dose ritonavir. It is relatively resistant to mutations that confer PI resistance and is in phase 3 trials in naïve-experienced patients and ART-experienced patients [73].

New class agents

CCR5 inhibitors

Maraviroc

Maraviroc, also known as UK-427 and 857, is a CCR5 antagonist. It blocks the CCR5 co-receptor on CD4 cells preventing HIV that uses this receptor from entering CD4 cells. It is manufactured by Pfizer and was approved for use by FDA on 24 April 2007. Furthermore, it was granted accelerated approval on 6 August 2007 for combination anti-retroviral treatment of adults infected only with detectable CCR5-tropic HIV-1, who have evidence of viral replication and who have HIV-1 strains resistant to multiple anti-retroviral agents [74]. Maraviroc is a substrate for CYP3A4 so has potential interactions with rifampicin, NNRTIs and PIs; levels of maraviroc are increased in patients also taking atazanavir, ritonavir-boosted lopinavir (Kaletra) and ritonavir-boosted saquinavir (Invirase) [59].

Vicriviroc (SCH-D) and aplaviroc (GSK-873140)

Vicriviroc is also a CCR5 antagonist and has a similar mechanism of action to maraviroc. It is being developed by Schering-Plough and is currently undergoing phase 2 clinical trials in treatment-experienced patients. Vicriviroc was discontinued in treatment-naïve patients because of poor virological outcomes. In one study, five patients in the vicriviroc arm developed malignancies, but the Drug and Safety Monitoring Board could not determine a causal link so the study was continued [59]. Phase 3 trials of GlaxoSmithKline's CCR5 antagonist, aplaviroc, were stopped in October 2005 following reports of severe liver toxicity [75].

TABLE 4

Potential benefits, toxicities and metabolism of new TB-HIV drugs in development

Drug name	Potential benefits	Potential for treating LTBI	Potential for shortening TB therapy	Metabolism and effect on CYP	Potential toxicities	Other
B drugs in development						
PA-824	Bacterio-cidal and bacterio-static, novel target reducing potential cross-resistance with existing drugs, treats MDR-TB	Yes	Unknown	No CYP metabolism		Requires activation by mTB F420 factor (Rv3547 enzyme)
OPC-67683	Novel target reducing potential cross-resistance with existing drugs, high MTB specificity, Treats MDR-TB	Yes	Yes	No CYP metabolism		Intracellular activity. Requires activation by Rv3547 enzyme
TMC 207/R207910	Novel target reducing potential cross-resistance with existing drugs, Bacterio-cidal and bacterio-static, Treats MDR-TB	Yes	Yes	Yes, CYP3A4		Synergistic with other TB meds esp. PZA
Moxifloxacin	Treats MDR-TB	Yes	Yes	Ciprofloxacin inhibits hepatic microsomal enzymes	GIT, hypersensitivity, CNS, hepatic necrosis, interstitial nephritis	Cross-resistance amongst quinolones
Gatifloxacin	Treats MDR-TB (weak data)		Yes	Ciprofloxacin inhibits hepatic microsomal enzymes	As above, dysglycaemia	Cross-resistance amongst quinolones
SQ-109	May treat ethambutol-resistant strains			CYP2D6 CYP2C19		
Drug name	Potential benefits	CYP metabolism/effect		Potential toxicities	Other	
HIV drugs in development						
Etravirine	Inhibits replication of wild-type and drug-resistant HIV			No safety concerns in phase 2 trials		
Darunavir	Retains efficacy against multi-PI-resistant viruses, higher genetic barrier to resistance	Metabolised by CYP				
Maraviroc	Active against diverse HIV-1 isolates from different clades, Does not compete with chemokine binding	Yes, substrate for CYP3A4		Mild/moderate Headache, dizziness, asthenia, flatulence, rhinitis	Immune modulation, escape mutants, altered viral tropism	
Aplaviroc	Trials discontinued	Yes, substrate for CYP3A4		Liver toxicity		
Vicriviroc	May be beneficial in salvage therapy—awaiting study conclusion			Poor virological outcomes in naïve patients		
Raltegravir	Virological benefit in salvage therapy	No (metabolised by glucuronidation)		Similar to placebo	Potential interaction with antibiotics	
Elvitegravir	Efficacy against MDR-HIV	Metabolised by CYP and glucuronidation, induces CYP3A		Similar to placebo	Potential interaction with antibiotics	

Integrase inhibitors: raltegravir and elvitegravir

Raltegravir, also known as MK-0518, is an integrase inhibitor and inhibits HIV–DNA integration into host DNA. It is being developed by Merck and is undergoing phase 3 clinical trials. Raltegravir's safety profile is comparable to placebo, and it has demonstrated virological benefit in salvage ART [76]. It is metabolised by glucuronidation (UGT1A1) and has no significant effect on CYP3A4 [59,76,77]. Elvitegravir, known as Gilead-9137, is also an integrase inhibitor and has a similar mechanism of action to raltegravir. Elvitegravir demonstrates efficacy against multi-drug-resistant HIV and has a side effect profile similar to placebo. It is metabolised by glucuronidation and CYP, so it has important drug interactions. It is also an inducer of CYP3A4. In clinical studies it is being used with ritonavir boosting to increase its half-life from three to nine hours, increase its AUC and prevent auto-induction of its metabolism [59,77,78].

Maturation inhibitor: PA-457

PA-457, also known as DSB, is a maturation inhibitor and blocks HIV maturation by disrupting cleavage of the capsid precursor. Panacos Pharmaceuticals is currently conducting phase 2 clinical trials. Data on the metabolism and potential toxicity of PA-457 are limited [59,79].

Future areas of research

Insight into how *M. tuberculosis* persists may illuminate novel ways to reduce the prolonged duration of TB therapy and emergence of drug resistance. In particular, there has been a recent advance in determining the bacillary determinants involved in adaptation to hypoxia: A state thought to be important in latent lesions [80]. It is also increasingly clear that metabolic adaptation to persistence involves a switch in metabolism to the use of fatty acids as a source of carbon. Deletion of both genes encoding isocitrate lyases 1 and 2 impairs the persistence of TB [81]. The absence of these gene products in humans may facilitate the development of glyoxylate cycle inhibitors as new drugs for the treatment of tuberculosis [81].

Given that novel anti-tuberculosis and anti-retroviral agents will be used concurrently in the future, structure–activity and structure–toxicity analyses combined with medicinal chemistry may address drug interactions at the design phase. *In vitro* screening systems for drug interactions with CYP enzymes and drug transporters are in use [82,83]. *In silico* prediction of adverse effects triggered by drugs metabolised by cytochrome P450 3A4 (metabolic transformations, drug–drug interactions) has been reported and may become more widespread at the discovery phase [84]. The clinical relevance of drug–drug interactions is best assessed in relevant patient populations. Population-based methods can be used during phases 2 and 3 of clinical drug development and in post-marketing evaluation to quantify known pharmacokinetic interactions and detect unanticipated interactions [85].

Conclusions

With the aid of philanthropic organisations, pharmaceutical companies and the TB alliance, numerous TB and HIV drugs with novel sites of action are currently in the drug development pipeline. Whilst phase 3 clinical trials still have to be conducted, these novel drugs hold the promise of improving combined TB–HIV therapy with the reduction of shared drug toxicities, drug resistance and long duration of TB therapy. It is our hope that these research ventures will impact those most afflicted by HIV and TB. All current labours will be in vain without the combined efforts of garnering political and societal commitment, significantly improving the infrastructure and adherence of DOTS and ART and making these new drugs financially available to the developing world.

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Novel Relationship between Tuberculosis Immune Reconstitution Inflammatory Syndrome and Antitubercular Drug Resistance

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(See the editorial commentary by Friedland on pages XXX–XXX)

Background. Tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS) is emerging as an important early complication of combination antiretroviral therapy in patients with TB in developing countries. The differential diagnosis of TB IRIS includes deterioration caused by other human immunodeficiency virus-related morbidities and drug-resistant TB.

Methods. We prospectively evaluated consecutive patients with suspected TB IRIS from February 2005 through July 2006 at a community-based secondary hospital in Cape Town, South Africa, by means of clinical case definitions for TB IRIS. Specimens were sent for TB culture and susceptibility testing, and a rapid test (FASTplaque-Response) was performed to expedite determination of rifampin susceptibility.

Results. One hundred patients with suspected TB IRIS were evaluated, 26 of whom were being retreated for TB. IRIS symptoms developed a median of 14 days (interquartile range, 7–25 days) after the initiation of combination antiretroviral therapy. In 7 patients, an alternative opportunistic disease was diagnosed. Rifampin-resistant TB was present in 13 patients, 9 of whom received a diagnosis after study entry (7 of 9 had multidrug-resistant TB). Undiagnosed rifampin-resistant TB was thus present in 10.1% of patients (95% confidence interval, 3.9%–16.4%) who presented with TB IRIS, once those with alternative diagnoses and TB with known rifampin resistance were excluded. In the remaining 80 patients, TB IRIS without rifampin resistance was the final diagnosis.

Conclusions. TB IRIS that is clinically indistinguishable from TB IRIS that occurs in the context of drug-susceptible disease may occur in patients with undiagnosed multidrug-resistant TB. Antitubercular drug resistance should be excluded in all cases of suspected TB IRIS, and corticosteroids should be used with caution for patients with presumed TB IRIS until the result of drug-susceptibility testing is known.

The scale-up of combination antiretroviral therapy (cART) in the developing world is progressing rapidly, improving survival among HIV-infected persons [1, 2].

An emerging complication of cART in countries with high rates of tuberculosis (TB) is TB immune reconstitution inflammatory syndrome (IRIS). “Paradoxical” TB IRIS manifests with new, worsening, or recurrent symptoms, signs, and/or radiological manifestations of TB after cART is initiated in patients receiving treatment for TB [3]. It occurs in 8%–43% of patients who initiate cART while receiving TB treatment [4–11] and is associated with exuberant antimycobacterial immune responses [12]. There is no diagnostic test for TB IRIS, and the differential diagnosis is wide, including failure of TB treatment attributable to antimicrobial resistance, or suboptimal antitubercular drug concentrations [13], drug reactions, or an alternative opportunistic condition. Published case definitions require that these be

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Table 1. Case definitions for tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS).

Criteria that must be met for the diagnosis of TB IRIS before the initiation of cART
Microbiologic, histologic, or very strong clinical evidence of TB
Initial improvement of ≥ 1 of the following during multidrug TB treatment: symptoms, Karnofsky score, weight, fever, clinical signs, or radiographic findings
The infecting strain of <i>Mycobacterium tuberculosis</i> is susceptible to rifampin (if this result is available)
The patient was receiving antitubercular therapy when cART was initiated
Criteria that must be met for the diagnosis of TB IRIS within 3 months after the initiation of cART
New or recurrent TB-related symptoms and/or
New or worsening TB manifestations, such as ≥ 1 of the following:
New or expanding lymph nodes
New or expanding tuberculous cold abscesses
New or expanding intracranial tuberculomas
New or expanding pulmonary infiltrates (radiographically confirmed)
New or recurrent tuberculous meningitis (after exclusion of bacteria and fungi)
New or enlarging serous effusions (pericardial, pleural, or ascitic; radiographically confirmed)
New or worsening granulomatous hepatitis
New or worsening granulomatous infiltration of bone marrow
Other new or worsening tuberculous lesions
No other opportunistic disease to explain the new or recurrent symptoms and/or new or worsening TB manifestations

excluded before a diagnosis of TB IRIS is made [14, 15]. TB IRIS can be severe and life-threatening, and there are anecdotal reports that suggest the use of adjunctive corticosteroid therapy [16, 17].

Concurrent with the increase in the prevalence of TB IRIS, the emergence of multidrug-resistant (MDR) and extensively drug resistant TB in settings in Southern Africa where HIV infection is prevalent has recently been highlighted [18, 19]. Determining the cause of deterioration in patients with TB during cART in resource-limited settings is important, because adjunctive corticosteroid therapy may worsen an already immunosuppressed patient's condition if used in the presence of incompletely efficacious TB treatment or other opportunistic infections. This study prospectively evaluated clinical case definitions of TB IRIS among 100 patients who were considered to have likely cases of TB IRIS. We found a high prevalence of unsuspected drug-resistant TB in this cohort, which has important implications for the diagnosis and management of this condition, as well as wider policy implications.

PATIENTS AND METHODS

Study site and participants. A prospective observational study was conducted under program conditions that involved 100 consecutive patients who were referred to GF Jooste Hospital (Cape Town, South Africa) with likely cases of TB IRIS. The TB incidence rate in the Western Cape province in 2006 was 1031 cases per 100,000 population [20], and the prevalence of antenatal HIV infection was as high as 33% [21]. More than 10,000 people have initiated cART within the catchment area of GF Jooste Hospital (M. Osler, Provincial Government of the

Western Cape, personal communication). The national TB program treats new TB cases with 6 months of therapy (rifampin, isoniazid, pyrazinamide, and ethambutol for 2 months, followed by rifampin and isoniazid for 4 months). The retreatment regimen includes the addition of streptomycin, as follows: 2 months of rifampin, isoniazid, pyrazinamide, ethambutol, and streptomycin; 1 month of rifampin, isoniazid, pyrazinamide, and ethambutol; and 5 months of rifampin, isoniazid, and ethambutol. Routine TB drug susceptibility testing (DST) is not performed for new TB cases. Patients receiving retreatment and patients not responding to TB treatment may have DST performed. DST was only performed for rifampin, isoniazid, and ethambutol during the study, in accordance with national guidelines.

First-line cART in South Africa is stavudine, lamivudine, and either nevirapine or efavirenz. Efavirenz is preferred for patients receiving rifampin-based TB treatment. Patients with a CD4 cell count < 200 cells/ μ L and/or World Health Organization stage 4 disease are eligible to commence cART.

Clinical case definitions of TB IRIS (table 1) were prepared, circulated, and discussed with participating primary care physicians. The definitions were in accordance with other published case definitions for IRIS [14, 15], in that they excluded patients with drug-resistant TB. Our case definitions, however, specifically used known resistance to rifampin as an exclusion criterion. We obtained information regarding initial TB diagnosis from the referral letter and by search of records from the regional laboratory, which processes all TB microbiologic specimens in our referral area. All patients aged ≥ 13 years who were referred to the hospital with suspected TB IRIS were in-

cluded. TB treatment adherence was assessed by self-report and on the basis of the patient's TB clinic card, on which each daily dose taken was documented with a tick. Patients who had <80% adherence to TB treatment reported were not included as patients with suspected TB IRIS. The Research Ethics Committee of the University of Cape Town approved this study (REC 337/2004).

Clinical assessment sought to exclude differential diagnoses based on clinical presentation. For example, for patients with respiratory symptoms, bacterial and pneumocystis pneumonia were investigated. Clinical specimens were sent for TB microscopic examination, culture, and DST at the time of presentation with suspected TB IRIS. DST for rifampin and isoniazid was performed at 2 nationally accredited laboratories. Both laboratories used the indirect proportion method: one laboratory used liquid media with the MGIT system, and the other used solid culture medium (Middlebrook 7H11 agar). For 36 patients, a rapid rifampin resistance assay was performed (FASTplaque-Response; Biotec Laboratories) [22]. The result of this assay was confirmed by culture-based DST, and the latter result is reported unless stated otherwise. A purified protein derivative enzyme-linked immunospot assay was performed, as described elsewhere [23]. Patients were classified as having TB IRIS if 2 clinicians agreed that, at initial assessment and during the follow-up period, the patient fulfilled at least 1 of the case definitions.

Statistical methods. Fisher's exact test was used to compare proportions, and the Mann Whitney *U* test was used to analyze differences between medians. The unpaired Student's *t* test with Welch's correction was used to compare enzyme-linked immunospot assay results.

RESULTS

One hundred patients (66 female and 34 male patients) with suspected TB IRIS were evaluated from February 2005 through July 2006. The median age was 31 years (interquartile range [IQR], 26–35 years), and the median baseline CD4 cell count was 50 cells/ μ L (IQR, 26–94 cells/ μ L). Twenty-six patients had received ≥ 1 course of prior TB treatment. Patients developed symptoms prompting referral with suspected TB IRIS a median of 14 days (IQR, 7–25 days) after starting cART. These patients were assessed during screening for a randomized placebo-controlled trial of prednisone for mild and moderate TB IRIS (ISRCTN 21322548). Thirty-eight patients were enrolled in that study, and 25 received corticosteroid treatment for TB IRIS outside that study, usually for severe TB IRIS. Final diagnoses are shown in figure 1.

Follow-up CD4 cell counts during the first year of cART were available for 77 patients. In 73 patients (95%), the follow-up CD4 cell count increased (median increase, 139 cells/ μ L; IQR, 64–241 cells/ μ L) from the pre-cART value. In 4 patients,

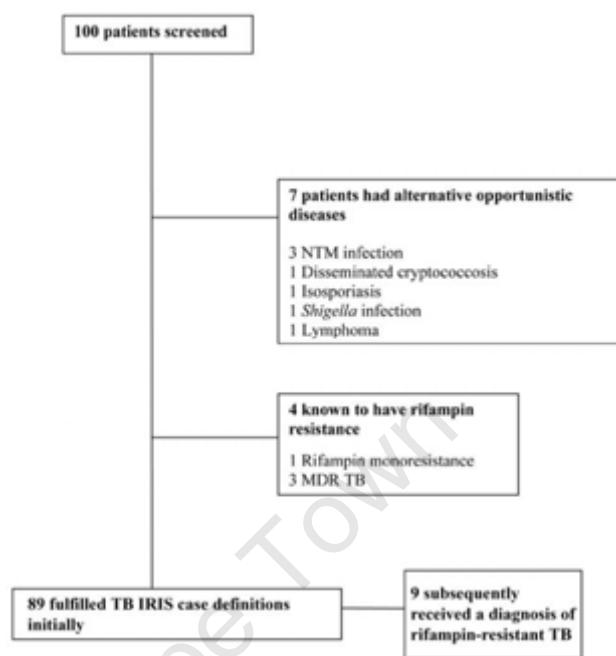


Figure 1. Final diagnosis for 100 patients with suspected cases of tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS). MDR, multidrug resistant; NTM, nontuberculous mycobacteria.

the CD4 cell count decreased by 1–50 cells/ μ L during the first year of cART. Follow-up viral loads, usually measured after 6 months of cART, were available for 74 patients. The viral load was <400 copies/mL in 65 patients, 400–1000 copies/mL in 5, and >1000 copies/mL in 4. In all 4 of these patients, the CD4 cell count increased by 77–365 cells/ μ L during cART.

Among the whole cohort, the initial TB diagnosis was made on the basis of culture of *Mycobacterium tuberculosis* in a clinical specimen for 41 patients and positive smear microscopy results for 31 patients. For 25 patients, a diagnosis of smear-negative or extrapulmonary TB was made on the basis of clinico-radiological data [24–26]. Although the other 3 patients were receiving TB treatment at presentation, the initial diagnosis of TB was incorrect, and they had nontuberculous mycobacterial infection.

Seven of the 25 patients with a clinico-radiological diagnosis had microbiological confirmation when they presented with suspected TB IRIS (4 were smear positive, and 3 were culture positive). For the other 18 patients, the diagnosis of TB was not microbiologically proven. These patients had TB symptoms and lymphadenopathy on abdominal ultrasound (5 patients), on chest radiograph (2), or peripherally (1); miliary infiltrates on chest radiograph (4); other radiographic pulmonary infiltrates (2); pericardial effusion (2); or pleural effusion (2). One of the patients who received a diagnosis on the basis of symptoms and abdominal nodes on ultrasound was subsequently found to have lymphoma and probably did not have TB.

Seven patients with suspected TB IRIS had clear evidence of an alternative opportunistic condition (figure 1). In 3 patients, this was a nontuberculous mycobacterial infection. These patients had experienced some symptomatic improvement while receiving TB treatment before initiation of cART and were then referred with suspected TB IRIS after commencing cART. Review of the initial sputum culture results revealed nontuberculous mycobacterial infection. In 2 of the 3 patients, nontuberculous mycobacteria were also cultured from blood samples at the time of assessment for suspected TB IRIS.

Twenty-five patients had DST performed at initial TB diagnosis. For 19 of these patients, the isolate was susceptible to rifampin and isoniazid; 2 had isolates monoresistant to isoniazid, 1 had an isolate monoresistant to rifampin, and 3 had MDR TB. In an additional patient (patient 3), DST of the isolate at TB diagnosis was performed retrospectively after presentation with suspected TB IRIS, and the isolate demonstrated monoresistance to rifampin (table 2). For this patient, the culture result at the time of presentation with TB IRIS was negative. All 4 patients who presented with suspected TB IRIS with known rifampin resistance were receiving appropriate therapy for MDR TB and reported at least partial clinical improvement before initiation of cART. These patients then presented with clinical deterioration 7–45 days after starting cART. Their clinical manifestations included fever or night sweats (2 patients), marked weight loss (2), new peripheral nodes (1), new pulmonary infiltrates on chest radiograph (2), and other recurrent TB symptoms. Although these features are typical of TB IRIS, the case definitions that we used excluded this diagnosis.

Eighty-five patients had at least 1 culture for *M. tuberculosis* performed during assessment for TB IRIS. Cultures for *M. tuberculosis* were performed on sputum (for 55% of patients), lymph node or abscess aspirate (18%), pleural fluid (8%), CSF (8%), or other clinical specimens (11%). Culture yielded *M. tuberculosis* for 17 patients (20%; 8 had MDR TB, 1 had isoniazid-monoresistant TB, and 7 had TB that was susceptible to rifampin and isoniazid). Drug susceptibility was undetermined for 1 patient (patient 6) (table 2), because the culture of the patient's sample yielded both nontuberculous mycobacteria and *M. tuberculosis*. This patient had a FASTplaque assay indicative of rifampin resistance. Including the patient whose initial isolate was rifampin resistant (patient 3) (table 2) and the patient with a FASTplaque result indicating rifampin resistance (patient 6) (table 2), 10 patients were found to have rifampin-resistant TB at the time of TB IRIS presentation. One of these patients was previously known to have MDR TB; thus, 9 (10.1%; 95% CI, 3.9%–16.4%) of 89 patients with suspected TB IRIS had rifampin-resistant TB that was previously unsuspected (table 2). All 9 of these patients reported symptomatic improvement after commencing standard TB treatment. For 1 of these patients (patient 8) (table 2), culture and DST at initial

TB diagnosis revealed susceptibility to rifampin and isoniazid, but repeat DST performed for suspected TB IRIS revealed MDR TB.

Table 3 compares the baseline characteristics of the 4 diagnostic groups. No statistically significant differences existed in univariate analysis, with the exception of shorter duration from initiation of TB treatment to initiation of cART in the group of patients who received diagnoses of TB IRIS with no rifampin resistance, compared with those who were known to have rifampin-resistant disease when they presented with suspected TB IRIS (median, 68 days vs. 199 days; $P = .02$).

Table 4 shows clinical, radiological, and laboratory features of the TB IRIS episode for the 80 patients who received a final diagnosis of TB IRIS without rifampin resistance. The TB IRIS case definitions that were fulfilled are also shown. Many patients fulfilled >1 case definition, and frequently, TB IRIS involved >1 organ system. The most frequent TB IRIS symptoms among these 80 patients were constitutional (68 patients; 85%), including night sweats, malaise, anorexia, and weight loss; respiratory (48; 60%); and abdominal (47; 59%), including abdominal pain, nausea, vomiting, and diarrhea. The symptoms, signs, and radiological findings for the 9 patients with rifampin-resistant TB diagnosed after presentation with suspected TB IRIS did not differ significantly from those of the patients who had TB IRIS without rifampin-resistant TB, with the exception of more frequent presence of lymphadenopathy on chest radiograph (7 of 9 patients vs. 30 of 80 patients; $P = .03$). Blood investigations were also similar between the 2 groups, although for the 9 patients with rifampin-resistant TB, the median C-reactive protein level was higher (179 mg/L [IQR, 100–212 mg/L] vs. 96 mg/L [IQR, 70–152 mg/L]; $P = .05$). Purified protein derivative enzyme-linked immunospot assay demonstrated evidence of immune activation in both groups, although the degree was less in the patients with rifampin-resistant TB (figure 2).

DISCUSSION

In Cape Town, the triple coincidence of very high TB case notification rates, an expanding epidemic of HIV infection, and the large-scale roll-out of cART has led to a large increase in the number of cases of TB IRIS (to date, there are hundreds of cases) presenting at our health care service. We prospectively evaluated clinical case definitions for TB IRIS for 100 cases; this was, to our knowledge, the largest TB IRIS case series reported to date. Once patients with alternative conditions (7 patients) and known rifampin-resistant TB (4 patients) were excluded, 9 (10%) of the remaining 89 patients were found to have rifampin-resistant TB only after presenting with suspected TB IRIS. This has important implications for the diagnosis and treatment of HIV-infected patients with TB whose conditions deteriorate after the introduction of cART.

Table 2. Cases of rifampin-resistant tuberculosis (TB) diagnosed after presentation with suspected TB immune reconstitution inflammatory syndrome (IRIS).

Patient	Age, years	Sex	Nadir CD4 cell count, cells/ μ L	Previous TB	Initial TB diagnosis	Course of events	TB IRIS manifestations
1	24	F	94	No	Pleural and pulmonary; not confirmed microbiologically	Symptoms improved and pleural effusion decreased while receiving Rif, INH, Pza, and Eth; started cART 8 weeks later; suspected TB IRIS onset 14 days later; sputum culture negative for TB twice at IRIS onset; sputum and pleural aspirate samples from 8–10 weeks after IRIS onset grew MDR <i>M. tuberculosis</i>	Recurrent constitutional, respiratory, and abdominal symptoms; fever and weight loss; cervical node enlargement, hepatomegaly; CXR showed progressively worsening nodular infiltrates and enlarging pleural effusion
2	29	F	16	No	Disseminated (pulmonary infiltrates on CXR and ascites on ultrasound); not confirmed microbiologically	Started on Rif, INH, Pza, and Eth; symptoms improved; started cART 13 weeks later; suspected TB IRIS onset 39 days later; bronchoscopy performed 8 weeks later (bronchial brushings grew MDR TB); thereafter, ongoing IRIS manifestations despite MDR TB treatment	High fevers, weight loss, and abdominal and constitutional symptoms; right hilar node enlargement on CXR with bronchial compression (on bronchoscopy); new right middle lobe infiltrate on CXR; enlarging cervical nodes
3	26	F	44	Yes	Disseminated; lymphadenopathy and hepatosplenomegaly on abdominal ultrasound; urine sample culture positive for <i>Mycobacterium tuberculosis</i> ; DST not requested at the time	Started Rif, INH, Pza, Eth, and Stm; reported 2-kg weight gain, and CRP level decreased (202 to 69 mg/L); started cART 1 month later; 5 days later, developed suspected TB IRIS; DST requested on initial urine isolate revealed Rif monoresistance	Abdominal pain, constitutional symptoms, and weight loss; progressively enlarging hepatomegaly, cervical node enlargement, para-umbilical cold abscess, peritonism, and marked tachycardia
4	51	F	35	No	Pulmonary; sputum sample cultured for <i>M. tuberculosis</i> ; DST not performed at the time	Symptomatic improvement while receiving Rif, INH, Pza, and Eth (cough and night sweats resolved, and patient felt stronger); started cART 2 months later; 7 days later, developed suspected TB IRIS; lymph node aspirate culture positive for MDR <i>M. tuberculosis</i>	Constitutional symptoms, marked weight loss, and respiratory symptoms; cervical node enlargement; CXR showed pulmonary infiltrates and enlarging thoracic nodes
5	31	F	3	No	Disseminated (CXR revealed pulmonary infiltrates and cervical adenitis); lymph node aspirate smear positive and sputum culture positive for <i>M. tuberculosis</i> ; no DST performed at the time	Initially improved while receiving Rif, INH, Pza, and Eth (night sweats resolved and general condition improved); then had chronic diarrhea followed by recurrent TB symptoms prior to cART; started cART 4 months after TB treatment; 3 days later, began deteriorating more rapidly, requiring admission; sputum culture positive for MDR <i>M. tuberculosis</i>	Recurrent cough; constitutional and abdominal symptoms; new pulmonary infiltrates with cavitation on CXR

(continued)

Table 2. (Continued.)

Patient	Age, years	Sex	Nadir CD4 cell count, cells/ μ L	Previous TB	Initial TB diagnosis	Course of events	TB IRIS manifestations
6	24	M	47	No	Disseminated; CXR revealed pulmonary infiltrates; abdominal ultrasound showed lymphadenopathy and splenic microabscesses; urine sample culture positive for <i>M. tuberculosis</i> ; no DST was performed at the time	Reported symptomatic improvement while receiving Rif, INH, Pza, and Eth; started cART 6 months after TB treatment; 29 days later, developed suspected TB IRIS; node aspirate grew <i>M. tuberculosis</i> and NTM; thus, formal DST could not be done; FASTplaque demonstrated Rif resistance	Constitutional symptoms, fever, new axillary lymphadenopathy, and tender hepatomegaly with jaundice
7	28	F	187	Yes ^a	Pulmonary (smear positive)	Started receiving Rif, INH, Pza, Eth, and Stm with some adherence lapses, but symptomatically better when started cART 5 months later; 7 days after starting cART, developed suspected TB IRIS; sputum culture positive for MDR <i>M. tuberculosis</i>	Fever and constitutional symptoms; pulmonary infiltrates on CXR
8	28	F	103	No	Disseminated; abdominal ultrasound showed lymphadenopathy and splenic microabscesses; sputum culture positive for <i>M. tuberculosis</i> ; susceptible to Rif and INH	Symptomatically improved while receiving Rif, INH, Pza, and Eth, with reported weight gain; 6 weeks after TB treatment, started cART; developed suspected TB IRIS 13 days later; then sputum culture positive for MDR <i>M. tuberculosis</i>	Respiratory, abdominal, and constitutional symptoms; weight loss, fever, and tachycardia; peritonism on examination; cervical node enlargement; CXR showed progressive pulmonary infiltrates and enlarging thoracic nodes
9	31	M	12	Yes ^a	Disseminated; CXR revealed pulmonary infiltrates and CSF smear positive for acid-fast bacilli; patient also received a diagnosis of cryptococcal meningitis before initiation of cART	Symptomatically improved while receiving treatment for TB and cryptococcal meningitis prior to cART; 48 days after starting cART, developed suspected TB IRIS; sputum culture positive for MDR <i>M. tuberculosis</i>	Respiratory and constitutional symptoms, weight loss, and headaches; hepatomegaly; CXR showed infiltrates with cavitation; CSF showed worsened lymphocytic meningitis that could have been due to TB or cryptococcal IRIS

NOTE. cART, combined antiretroviral therapy; CRP, C-reactive protein; CXR, chest radiograph; DST, drug susceptibility testing; Eth, ethambutol; INH, isoniazid; MDR, multidrug resistant; NTM, nontuberculous mycobacteria; Pza, pyrazinamide; Rif, rifampin; Stm, streptomycin.

^a Previously defaulted TB treatment.

In the 9 patients with rifampin-resistant TB, presentation was suggestive of TB IRIS, with improvement while receiving TB treatment before initiation of cART and then deterioration during the weeks after the initiation of cART. The obvious question is whether the condition of the patients with drug-resistant TB deteriorated because of suboptimally treated TB, TB IRIS, or both? Our case definitions, which are similar to those used by other researchers [14, 15], classified known rifampin resistance as excluding TB IRIS. However, in light of our observations, we propose that antitubercular drug resistance and TB IRIS are not mutually exclusive and may overlap in the same person. First, given that TB IRIS immunopathology

is attributable to restored antigen-specific immunity to *M. tuberculosis* antigens [12], it is reasonable to conclude that TB IRIS may occur in response to drug-susceptible or drug-resistant strains, whether the latter are treated or untreated. The antigen stimulus for TB IRIS is unlikely to differ in these scenarios.

Second, in the 4 patients with known rifampin-resistant TB, all improved while receiving treatment for MDR TB, and then their conditions deteriorated after cART initiation. The deterioration of their conditions was most likely attributable to TB IRIS, given the timing. These patients may be at higher risk of TB IRIS than patients with rifampin-susceptible TB. *M. tuber-*

Table 3. Characteristics of the cohort.

Characteristic	Patients			
	Received a diagnosis of TB IRIS without Rif resistance (n = 80)	Presented with suspected TB IRIS and then received a diagnosis of Rif-resistant TB (n = 9)	Known to have Rif-resistant TB at presentation with suspected TB IRIS (n = 4)	Presented with suspected TB IRIS and then received a diagnosis of an alternative OD (n = 7)
Female sex	52 (65)	7 (78)	1 (25)	6 (86)
Age, years	31 (27–36)	29 (26–31)	31 (28–35)	30 (24–34)
Previous TB	18 (23)	3 (33)	2/3 (67)	3 (43)
Prior cART ^a	4 (5)	1 (11)	1 (25)	0 (0)
WHO clinical stage ^b				
3	28 (35)	2 (22)	0 (0)	2 (29)
4	52 (65)	7 (78)	4 (100)	5 (71)
cART regimen at assessment				
d4T, 3TC, and EFV	64 (80)	8 (89)	4 (100)	6 (86)
Other	16 (20)	1 (11)	0 (0)	1 (14)
TB disease form ^c				
Pulmonary	36 (45)	3 (33)	2 (50)	3 (43)
Extrapulmonary	44 (55)	6 (67)	2 (50)	4 (57)
Time from start of TB treatment to start of cART, days	68 (35–93)	84 (47–115)	199 (121–447)	69 (21–125)
Time from start of cART to TB IRIS onset, days	14 (8–23)	13 (7–29)	35 (17–45)	35 (7–52)
Baseline CD4 cell count, cells/ μ L	50(26–94) ^d	44(16–94)	84 (55–184)	72 (13–85)
Baseline viral load measured	61 (76)	5 (56)	0 (0)	5 (71)
Baseline viral load, value $\times 10^5$ copies/mL	2.1 (9.8– 5.0)	1.7 (1.4–5.6)	...	2.5 (1.1–5.0)

NOTE. Data are no. (%) of patients or median value (interquartile range). cART, combination antiretroviral therapy; d4T, stavudine; EFV, efavirenz; IRIS, immune reconstitution inflammatory syndrome; OD, opportunistic disease; Rif, rifampin; TB, tuberculosis; 3TC, lamivudine; WHO, World Health Organization.

^a Excludes patients who received single-dose nevirapine for prevention of mother-to-child transmission.

^b WHO staging includes current TB diagnosis.

^c The pulmonary TB category includes patients who had pulmonary TB only, and the extrapulmonary TB category includes patients with extrapulmonary TB only and those with both pulmonary TB and extrapulmonary TB.

^d Seventy-nine of these 80 patients had a baseline CD4 cell count available.

culosis bacillary load has been suggested as a risk factor for the condition [7], and even those patients who are effectively treated for MDR TB are likely to have slow bacillary clearance. This may partially account for our observation that these 4 patients developed TB IRIS despite a long interval between initiation of TB therapy and initiation of cART (table 3).

Third, 9 patients had undiagnosed rifampin-resistant TB when they presented with suspected TB IRIS. These patients all reported at least partial symptomatic response to standard TB treatment before the initiation of cART. It is possible for patients with MDR TB to initially respond to first-line TB treatment [27–29], either because the organism is sensitive to ethambutol and pyrazinamide or because the patient is dually infected with MDR TB and a susceptible strain [30]. In addition, patients may improve while receiving treatment for drug-susceptible TB and then be reinfected with drug-resistant TB, or the infecting organism may develop rifampin resistance during treatment. Either of these scenarios could have occurred for patient 8 (table 2). For these 9 patients who received a diagnosis of rifampin-resistant TB after presenting with suspected TB IRIS, symptomatic deterioration occurred 3–48 days after the initiation of cART—the characteristic timing of TB IRIS. The clinical and radiological features of these patients

when they presented with suspected TB IRIS, with the exception of more frequent presence of lymphadenopathy on chest radiograph, were not significantly different from those of patients with TB IRIS with no drug resistance. We propose that TB IRIS exacerbated undiagnosed drug-resistant TB. Support for the idea that TB IRIS was present in this group comes from the enzyme-linked immunospot assay data, which showed expansions of purified protein derivative-specific IFN- γ -producing T cells (which are a reported characteristic of TB IRIS [12]), irrespective of drug susceptibility. The overlap of IRIS and drug resistance with respect to cryptococcal infection has been highlighted elsewhere [31].

Seven patients with suspected TB IRIS had alternative opportunistic diseases that explained their clinical deterioration. Patients with advanced immunosuppression may have multiple opportunistic conditions. A thorough examination for alternative infections and malignancies before diagnosis of TB IRIS is essential. Three patients receiving TB treatment at their local clinic actually had nontuberculous mycobacterial infection when the result of the original culture was followed up; this underscored the importance of reviewing the original TB diagnosis during the assessment of these patients. The performance of these case definitions may not be the same in settings

Table 4. Clinical, radiographic, and laboratory features of tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS) in 80 patients who received a diagnosis of TB IRIS without rifampin resistance.

Variable	Patients
TB IRIS case definitions fulfilled	
New, recurrent, or worsening symptoms	80 (100)
New or worsening TB manifestations	59 (74) ^a
New or expanding lymph nodes	35 (44)
New or expanding cold abscesses	3 (4)
New or expanding intracranial tuberculomas	1 (1)
New or expanding pulmonary infiltrates	22 (28)
New or recurrent TB meningitis ^b	6 (8)
New or enlarging serous effusions	13 (16)
New or worsening granulomatous hepatitis	2 (3)
New or worsening granulomatous bone marrow infiltrate	5 (6)
Other new or worsening TB lesion ^c	3 (4)
Other physical signs	
Fever (temperature, >37.4°C)	31 (39)
Hepatomegaly	45 (56)
Splenomegaly	7 (9)
Peritonism	4 (5)
New neurological signs	7 (9)
Radiographic features	
On chest radiograph	
Pulmonary infiltrates	52/80 (65)
Hilar/mediastinal nodes	30/80 (38)
Pleural effusion	17/80 (21)
On ultrasound	
Abdominal nodes	27/35 (77)
Ascites	10/35 (29)
Pericardial effusion	10/35 (29)
Focal splenic lesions	7/35 (20)
On CT: head lesions	3/8 (38) ^d
Laboratory features	
Total bilirubin level, $\mu\text{mol/L}$ ($n = 13$) ^e	54 (16–103) [0–21]
Alkaline phosphatase level, IU/L ($n = 56$)	181 (103–326) [40–120]
Alanine aminotransferase level, IU/L ($n = 62$)	41.5 (28–59.8) [5–40]
γ -Glutamyl transferase level, IU/L ($n = 15$)	217 (152–482) [0–35]
Hemoglobin level, g/dL ($n = 73$)	9.1 (7.8–10) [M: 13.0–17.0; F: 12.0–15.0]
WBC count, value $\times 10^9$ cells/L ($n = 71$)	5.4 (3.7–8.7) [4.0–10.0]
Platelet count, value $\times 10^9$ cells/L ($n = 65$)	362 (268–462) [178–400]
C-reactive protein level, mg/L ($n = 72$)	96 (70–152) [0–10]
C-reactive protein level >10 mg/L ($n = 72$)	71 (99)

NOTE. Data are no. (%) of patients or median value (interquartile range) [reference range].

^a The other 21 patients fulfilled only the case definition relating to new, worsening, or recurrent symptoms.

^b TB IRIS meningitis was defined as ≥ 5 lymphocytes and/or polymorphs in a CSF sample from a patient with new or recurrent meningism or other new neurological symptoms after exclusion of bacterial and fungal meningitis.

^c The other manifestations were arthritis (in 2 patients) and bursitis (in 1 patient).

^d Eight patients had CT performed: tuberculomas were shown for 1 patient and a new infarct (in 1 patient) and hydrocephalus (in 1 patient) were shown for the others.

^e Bilirubin level measurement was performed mainly for those patients in whom jaundice was clinically apparent. Nine of the 13 patients who had their bilirubin level measured had a total bilirubin level >21 $\mu\text{mol/L}$.

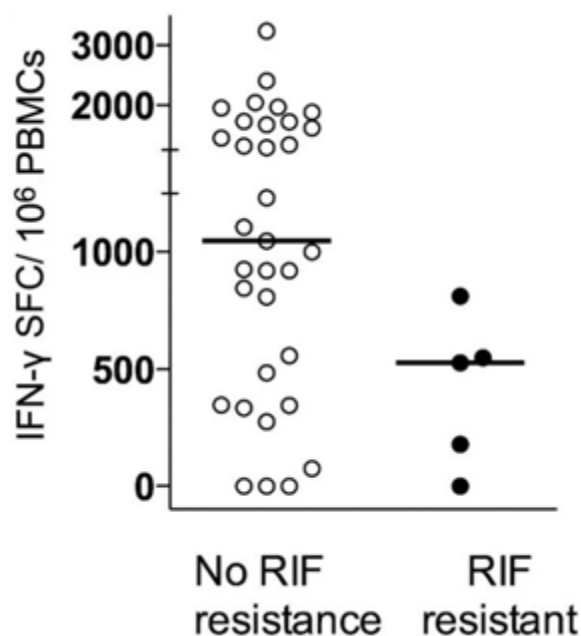


Figure 2. Comparison of purified protein derivative enzyme-linked immunospot assay results for 33 patients with tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS) with no rifampin (RIF) resistance (median, 1047 spot-forming cells [SFC] $\times 10^6$ PBMCs; interquartile range, 417–1700 SFC $\times 10^6$ PBMCs) with those for 5 patients who received a diagnosis of RIF-resistant TB after presenting with TB IRIS (median, 529 SFC $\times 10^6$ PBMCs; interquartile range, 89–680 SFC $\times 10^6$ PBMCs; $P = .04$).

with lower TB incidence rates, where patients might more frequently present with other reasons for deterioration.

Frequently, patients had signs, symptoms, and radiological manifestations suggestive of multiple organ system involvement with TB IRIS. This may be explained by profound immunosuppression (median nadir CD4 cell count, 50 cells/ μ L), predisposing to disseminated TB. The most frequent organ systems involved were the respiratory system (respiratory symptoms and chest radiograph infiltrates) and, hitherto little appreciated, the abdominal organs. Of the 80 patients with TB IRIS with no rifampin resistance, 59% had abdominal symptoms; 56% had hepatomegaly, 9% had splenomegaly, and 5% had peritonism. Abdominal nodes were present in 77% of those who underwent ultrasonography. Liver function derangement, particularly a cholestatic pattern, was common. These findings suggest that, if patients develop abdominal symptoms or liver function derangement after commencing cART, TB IRIS should be considered in the differential diagnosis in addition to drug-related adverse effects, such as pancreatitis, lactic acidosis, and drug-induced hepatitis.

There were several limitations to our study. For most patients, the initial TB diagnosis had been made in primary care according to program guidelines, and only 41% of the cases

were confirmed by culture. Most patients also initiated cART at the same facilities; thus, examination and radiographic data were incomplete. All patients had a chest radiograph performed to investigate TB IRIS, but few were performed at the time of cART initiation. In addition, patients who are smear positive do not routinely undergo chest radiography at TB diagnosis. Therefore, for many patients with symptoms of TB IRIS who had radiographic pulmonary infiltrates (65% had infiltrates), it was impossible to determine whether these were new or expanding, which potentially underestimated the number of cases fulfilling the pulmonary infiltrate case definition and, similarly, the serous effusion case definition. Many patients had hepatomegaly (56% of patients) with cholestatic liver derangement, which suggested granulomatous hepatitis [32], but this was only confirmed in 2 patients, because severity otherwise was insufficient to warrant a biopsy. The number of cases fulfilling the granulomatous hepatitis case definition might, for this reason, also be underestimated. It is for these reasons that the diagnosis of TB IRIS was made exclusively on the basis of a clear history of symptom improvement before the initiation of cART, followed by new, worsening, or recurrent TB symptoms after the initiation of cART in many patients (26% of patients). Follow-up CD4 cell count and HIV viral load measurements during cART were unavailable for one-quarter of the patients. These tests are performed every 6 months during cART under program conditions; however, many patients were transferred to other facilities, and some were lost to follow-up or died by 6 months. Determination of viral load or CD4 cell count at the time of TB IRIS diagnosis did not, however, form part of our case definitions. For patient 6 (table 2), the FASTplaque assay demonstrated rifampin resistance. This could not be confirmed by culture-based DST. The presence of nontuberculous mycobacterial coinfection in the patient's specimen may have affected the FASTplaque result, but it is worth noting that the specimen was culture positive for *M. tuberculosis* after 7 months of TB treatment; this supported a diagnosis of rifampin-resistant TB.

Corticosteroids have been proposed as a treatment for TB IRIS, although the only evidence currently available is anecdotal [17]. There are potential risks of corticosteroid treatment for HIV-infected patients with TB, including herpes virus reactivation and Kaposi sarcoma [33, 34]. There is no present clinical trials evidence supporting the use of corticosteroids for the treatment of TB IRIS, other than for TB meningitis and, perhaps, pericardial disease (neither with evidence in the context of cART [35, 36]). Assessment of suspected TB IRIS should trigger re-evaluation of the TB diagnosis and the adequacy of antimicrobial therapy. Such assessment should occur before the instigation of corticosteroid therapy; otherwise, there is a risk that patients who remain deeply immunosuppressed (despite IRIS) may receive steroid therapy without adequate antimicro-

bial coverage. Our study highlights the pressing need to develop and implement rapid techniques to diagnose drug resistance that are appropriate to resource-limited conditions. The use of the FASTplaque assay in the present study was an attempt to overcome this problem, and there are encouraging data emerging from other studies of rapid tests [37]. Overall, our data also support the use of routine DST for HIV-infected patients with TB.

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6

HIV–TB Drug Interactions¹⁾

Tolu Oni, Dominique J. Pepper, and Robert J. Wilkinson

6.1

Important Concepts and Definitions [1, 2]

- **Combination antiretroviral therapy** Antiretroviral therapy where typically three or four drugs, in most cases from different drug classes, are given in combination.
- **Cytochrome P450 (CYP)** Membrane-associated hemoproteins, located either in the inner membrane of the mitochondrion or in the endoplasmic reticulum of the cells. CYPs metabolize endogenous and exogenous compounds, such as hormones (estrogen and testosterone) and xenobiotics.
- **P-glycoprotein** An energy-dependent efflux pump that exports substrates out of the cell, and is expressed in the epithelial cells of the gastrointestinal tract, the liver, the kidneys, the blood–brain barrier, and in CD4 + lymphocytes.
- **Drug interaction** Said to occur when the disposition of one drug is altered by another.
- **Pharmacokinetic drug interaction** Involves alteration in absorption, transport, distribution, metabolism or excretion of a drug, the results of which can be a decreased or an increased exposure, leading to reduced efficacy or increased toxicity, respectively.
- **Pharmacodynamic drug interaction** Pharmacological response to the drug is directly altered, leading to potentiation of effect (including toxicity) in either an additive or synergistic manner, or antagonism.

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6.2

Background

As of December 2007, two billion people globally are infected with *Mycobacterium tuberculosis*-mediated tuberculosis (TB) [4], and 33.2 million with human immunodeficiency virus (HIV) [5]. Where these pandemics intersect, they are the most common cause of death among young adults in many countries. In 2007, 2.1 million AIDS-related deaths occurred worldwide [5], while in 2006 1.7 million deaths were attributed to TB [6]. The developing world accounts for the majority of the 11 million people coinfecting with HIV and TB (HIV-TB) [4]. In 2006, there were an estimated 709 000 new HIV-infected TB cases, 85% of which occurred in Africa [6]. TB and HIV are inexorably entwined; the annual incidence of TB disease doubles within the first year of HIV infection [7], and may reach 30% per annum in the profoundly immune-suppressed [8]. Likewise, TB disease synergistically accelerates the progression of HIV infection to acquired immune deficiency syndrome (AIDS), by promoting viral replication in immunologically activated CD4 cells [9–11]. Soon after the commencement of TB therapy, combination antiretroviral therapy (cART) is often required to ameliorate the profound immune suppression of AIDS. In addition, simultaneous treatment(s) for associated AIDS-defining illnesses and complications is/are often necessary. While dual HIV-TB therapy appears associated with improved survival compared to delayed antiretroviral therapy [12], several substantial challenges exist, namely drug–drug and drug–disease interactions, shared drug toxicities, immune reconstitution inflammatory syndrome (IRIS), and high pill burdens [3]. In particular, the possibility for drug–drug interactions that have clinically important consequences increases in HIV-infected patients receiving cART if three or more comorbid illnesses occur simultaneously, or three or more antiretroviral agents are taken concurrently [13]. In this chapter we describe the current therapy for HIV-TB, and discuss the clinically relevant drug–drug and drug–disease interactions that may occur in HIV-infected patients receiving dual HIV-TB therapy. A prediction is also made of the potential drug interactions that are likely to occur in the developed world.

6.3

Current Therapy for Tuberculosis and HIV

Tuberculosis therapy is a multidrug regimen prescribed for a minimum of six months. Multidrug treatment needs to be prolonged as single-agent TB therapy rapidly gives rise to drug-resistant organisms [14]. The advent of rifampin and pyrazinamide allowed the development of highly effective “short-course” TB regimens – usually a two-month intensive phase with rifampin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E) – followed by a four-month continuation phase with RH (2RHZE/4RH) [15]. Multi-drug resistant (MDR) TB, which is defined as TB resistant to RH, is treated with less-effective agents for up to 18 months after sputum conversion. Treatment is often initially empiric whilst awaiting drug

susceptibility results, and later individualized when such results are known. Regimens may last for up to 24 months and include (according to susceptibility profile): pyrazinamide, ethambutol, terizidone, ethionamide, an oral fluoroquinolone (ofloxacin/ciprofloxacin/gatifloxacin/moxifloxacin), and an injectable agent (kanamycin/amikacin/capreomycin). Extensively drug-resistant (XDR) TB, defined as MDR TB with resistance to both an oral fluoroquinolone and an injectable agent [16], also requires individualized treatment regimens [17, 18]. In addition, drugs to which *M. tuberculosis* remains susceptible, such as capreomycin, *para*-aminosalicylic acid (PAS), co-amoxycylav and linezolid, may be added.

Combination antiretroviral therapy (cART) is a lifelong, multidrug regimen. In patients infected with HIV, millions of virions are produced daily, and the reverse transcriptase target rapidly mutates. Hence, initial therapies with single or dual nucleoside reverse transcriptase inhibitors (NRTIs), such as zidovudine (AZT) and didanosine (ddI), were only partially effective and rapidly led to viral drug resistance [19]. Effective therapy only became possible when non-nucleoside reverse transcriptase inhibitor (NNRTI) and viral protease inhibitor (PI) drugs were developed. Combinations of these three drug classes may lead to a prolonged suppression of HIV replication, and ultimately to a degree of immune recovery. Adherence to cART is crucial for successful viral suppression, which is very closely related to immune restoration and survival [20].

6.4

Potential Drug–Drug and Drug–Disease Interactions

Numerous concurrent diseases occur in profoundly immune-suppressed HIV-TB patients, including *Pneumocystis jiroveci* pneumonia, toxoplasmosis, Kaposi's sarcoma, deep-vein thrombosis, seizure disorders, and sepsis [21]. The potential for drug–drug and drug–disease interactions increases when treatment for these illnesses is prescribed concomitantly with HIV and TB therapy (Figure 6.1). While cART partially restores immune function and prolongs life, HIV-infected patients are at increased risk of metabolic and vascular disorders; these disorders may occur as a direct result of HIV infection [22], as side effects of HIV therapy [23–25], or because prolonged survival allows HIV-infected patients to develop diseases that would otherwise have occurred later in life. Simultaneous treatment for HIV, diabetes mellitus and diseases due to atherosclerosis, will challenge many clinicians, especially as a subgroup of patients will still have an increased risk of TB disease [26] and require treatment with rifamycins.

Potential drug–drug and drug–disease interactions should always be considered in patients infected with HIV-TB, especially as many of the drugs used to treat HIV-TB and comorbid diseases are either inducers, inhibitors, or substrates of cytochrome p450 (CYP). Drugs that induce a particular CYP isoenzyme increase the rate at which the CYP isoenzyme metabolizes its substrate to metabolites. Conversely, inhibitors decrease the rate of metabolite production, resulting in increased substrate. Rifampin is a potent inducer of CYP3A4, whereas ritonavir is an inhibitor of CYP3A4.

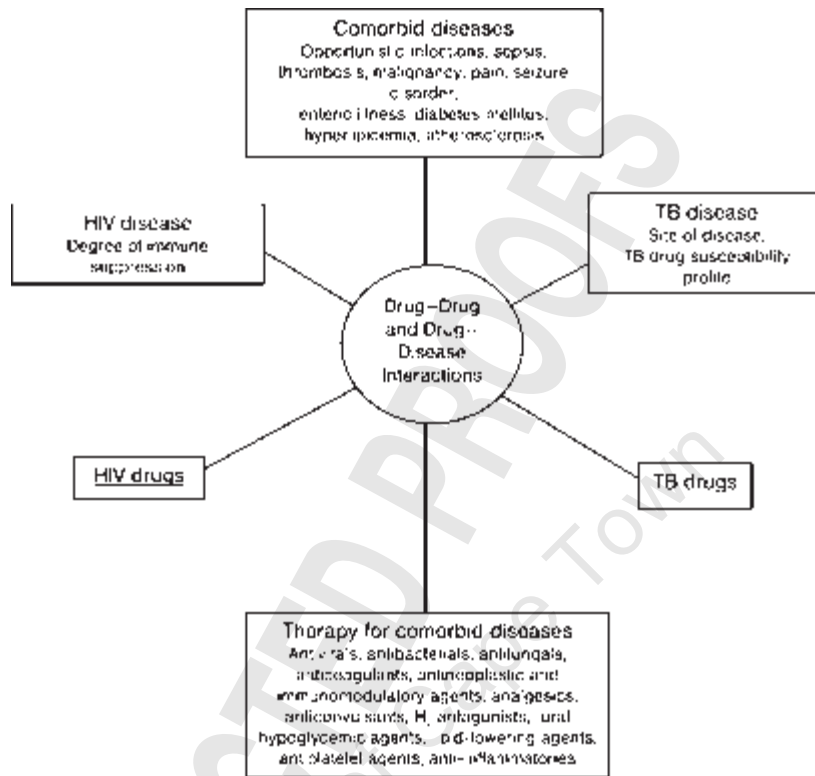


Figure 6.1 Potential contributors to HIV-TB drug-drug and drug-disease interactions: TB, HIV and comorbid diseases and therapy.

When warfarin and oral contraceptives are coadministered with CYP inducers, suboptimal anticoagulation and contraceptive failure, respectively, may occur. Similarly, when statins, calcium antagonists and benzodiazepines are coadministered with CYP inhibitors, rhabdomyolysis, symptomatic hypotension and excessive sedation, respectively, may result.

6.5

Treatment of Tuberculosis

Combination TB therapy with PAS and streptomycin was first reported in 1950 [27], it having been recognized at an early stage that monotherapy rapidly gave rise to bacterial resistance. With the later advent of rifampin, isoniazid, and pyrazinamide, large studies demonstrated that the best chance of curing *M. tuberculosis* would be provided by the combined use of rifampin and isoniazid for six months, with pyrazinamide and ethambutol added for the first two months. Rifampin results in a faster sputum conversion [28] and a shorter treatment duration [29]. The use of

1 rifampin in a multidrug regimen reduces the emergence of drug-resistant strains.
 2 In order to optimize pharmacotherapy, factors affecting the absorption, distribu-
 3 tion, metabolism and elimination of anti-tuberculosis drugs should be considered,
 4 as should their pharmacokinetics and significant interactions (see Table 6.1
 5 and below).
 6

7 6.5.1

8 **Rifampin**

9
 10 As rifampin interacts with a wide range of drugs, it is useful to understand its
 11 pharmacokinetics in order to predict possible drug interactions. An important
 12 mechanism of drug interactions with rifampin is the induction of drug-metabo-
 13 lizing enzymes such as CYP 3A4 [30] in the small intestine and liver. Rifampin also
 14 induces the CYP 2C isoenzymes, and therefore has the potential for interaction with
 15 CYP 2C substrates, including sulfonylureas such as gliclazide [31]. However, other
 16 possible mechanisms have been sought, as not all drug interactions can be
 17 explained by an induction of the cytochrome P450 system (see Table 6.1). In
 18 particular, the ATP-binding cassette (ABC) efflux transporter P-glycoprotein, located
 19 in the apical membrane of enterocytes, has been found to have a role in the
 20 elimination and bioavailability of certain drugs [32, 33]. Rifampin has been shown
 21 to be an inducer of P-glycoprotein, and this represents another mechanism through
 22 which drug–drug interactions can occur; indeed, this is thought to be the mech-
 23 anism for rifampin–digoxin drug interaction [34]. Such induction is thought to be
 24 tissue-specific, as renal P-glycoproteins do not appear to be induced by rifampin [34],
 25 possibly because only enterocytes are locally exposed to high concentrations of orally
 26 administered drug, and not the kidneys or liver. In addition, it was found that
 27 human MRP2 (part of the multidrug-resistant protein family, a member of the ABC
 28 transporters, and expressed also in small intestine enterocytes) is induced by
 29 rifampin, thus alluding to another possible mechanism for drug interaction of
 30 rifampin with other drugs [35].

31 Rifampin may also activate the human glucocorticoid receptor by acting as a ligand
 32 and binding to the receptor, and is therefore a potential immunosuppressive [36];
 33 however, this point has attracted controversy [37]. Rifampin may reduce the con-
 34 centration of drugs metabolized by uridine diphosphate glucuronosyl transferase
 35 (UDPGT) and sulfotransferase (e.g., moxifloxacin) [38].

36 *Trimethoprim-sulfamethoxazole* is commonly used in HIV-infected patients as
 37 prophylaxis against *Pneumocystis jiroveci* pneumonia (PJP) and toxoplasmosis. In
 38 one clinical study it was suggested that rifampin could reduce the efficacy of co-
 39 trimoxazole as prescribed for toxoplasmosis prophylaxis [39]. A pharmacokinetic
 40 study conducted subsequent to this study showed reduced concentrations of both
 41 trimethoprim and sulfamethoxazole in the presence of rifampin, with the suggested
 42 mechanisms being rifampin's induction of either the CYP450 system, the induction
 43 of UDPGT, or by the induction of hepatic acetylation of sulfamethoxazole [40]. The
 44 effect on clinical outcome of this reduction in plasma concentration, however, is not
 45 known. Data are also available suggesting that rifampin levels increase after the

Table 6.1 Pharmacokinetics of TB drugs.

Drug name	CYP			P-glycoprotein		Drug metabolism (other than CYP)	Percentage (%) protein binding, principal protein bound to
	Substrate	Inducer	Inhibitor	Substrate	Inducer		
First-line TB drugs							
Rifampin	No	3A4, 1A2, 2C, 2D6			Yes	Metabolized by deacetylation induces UDPGT and sulfotransferase	85% protein bound
Rifapentine		3A4, 2C8/9					97% protein bound
Rifabutin	3A	3A, 2D					Least protein bound -70%
Isoniazid	No	2E1	2C9, 2C19			Metabolized by NAT2 and 2E1 Inhibits MAO	
Pyrazinamide	No	No				Metabolite inhibits uric acid secretion by renal tubules	
Ethambutol	No	No				15% metabolized to aldehyde and dicarboxylic metabolites	
Streptomycin	No	No	No			No identified metabolites	

Second-line drugs	No	No	No	No identified metabolites
Kanamycin	No	No	No	No identified metabolites
Amikacin	No	No	No	No identified metabolites
Ofloxacin			Yes	
Ciprofloxacin [85]			Yes	
Moxifloxacin [86]			Yes	
Ethionamide	3A			20–40% 40–50%
Capreomycin				
Clarithromycin [87–89]	3A	3A1		Hydroxylation and oxidative N-demethylation >50% acetylated
PAS [90]				
Augmentin				
Linezolid [79, 91]				Metabolized by nonenzymatic oxidation, reversible inhibitor of MAO A/B 31%, albumin
Cycloserine				
Terizidone				

Abbreviations: CYP: cytochrome p450 isoenzyme; PAS: *para*-aminosalicylic acid; TB: tuberculosis; UDPGT: uridine diphosphate glucucosyltransferase.

administration of co-trimoxazole [41] but, again, the clinical significance of this is unknown.

Dapsone is another drug used in the prophylaxis of PJP. It has been suggested that rifampin decreases the serum concentration of dapsone through the induction of CYP3A4 and, indeed, one study has shown dapsone clearance to be increased by between 69% and 122% [42]. It was not clear whether this reduction in concentration would result in a decreased efficacy in clinical practice [43], though some experts believed this to be the case [44]. Rifampin is itself metabolized by deacetylation, and is therefore unaffected by the CYP450 system; it is known to be capable of inducing its own metabolism, however [45].

6.5.2

Rifapentine

Rifapentine is a long-acting cyclopentyl-derivative of rifampin, and is an inducer of CYP3A4 and CYP2C8/9 of the same order of magnitude as rifampin [46]. Rifapentine does not possess autoinductive properties [47] after repeated administration, however. In the plasma, rifapentine has been shown to be highly protein-bound (97–99%), primarily to albumin, in healthy volunteers [48]. Such protein binding may be important for the drug's pharmacodynamics when compared to rifabutin, which is the least protein-bound rifamycin and shows little reduction in efficacy with dose reduction [49].

The few drug interaction studies performed with rifapentine have shown the most significant interaction to be with indinavir; coadministration of the two drugs led to a reduction of 55% in the maximum plasma concentration, and of 70% in the area under the concentration–time curve (AUC) [50].

6.5.3

Rifabutin

Rifabutin not only induces but is also metabolized by CYP 3A4; this results in complex interactions with inhibitors of CYP450, such as protease inhibitors, anti-fungal agents, and macrolide antibiotics. Rifabutin is also a potent autoinducer. Some data are available suggesting that microsomal cholinesterase is also involved in the metabolism of rifabutin [51].

6.5.4

Isoniazid

Isoniazid inhibits CYP isoenzyme systems and monoamine oxidase (MAO), and so is associated with some drug interactions [52]. Isoniazid is mainly metabolized by hepatic *N*-acetyltransferase 2 (NAT2) and CYP450 2E1. Unlike the rifamycins, it is mostly excreted via the urine. It is believed that isoniazid kills the largest population of *M. tuberculosis* in the rapidly growing phase. Several studies have shown that the acetylator status of individuals may play a role in determining clinical outcome, with

slow acetylators showing a reduced enzyme activity. Despite this, it has been shown that when isoniazid is given at least twice-weekly, the clinical outcome is independent of acetylator status, although slow acetylators are more prone to hepatotoxicity [53]. These results have led some to suggest that NAT2 genotyping might be used to ascertain acetylator status in the monitoring of TB treatment [54]. Acetylator status appears relevant in isoniazid's interaction with paracetamol (acetaminophen), with some data showing rapid acetylators to have an increase in the levels of paracetamol metabolites. It is thought that the induction of the CYP450 system leads to an increase in hepatocellular injury due to an increased formation of toxic metabolites of paracetamol [55]. A study conducted in mice showed that aspirin antagonized isoniazid treatment, with possible implications for the coadministration of salicylate-based anti-inflammatories and isoniazid.

6.5.5

Pyrazinamide and Ethambutol

Pyrazinamide and ethambutol each have a limited drug interaction profile [56].

The main metabolite of *pyrazinamide*, pyrazinoic acid, inhibits the renal tubular secretion of uric acid and hence may induce hyperuricemia. There is a scarcity of data regarding possible drug interactions of pyrazinamide; hepatotoxicity following its administration has also been demonstrated, although the mechanism involved is not clear [57]. An allopurinol–pyrazinamide interaction has been reported that causes a build-up of pyrazinoic acid and reduces the renal secretion of uric acid [58].

Ethambutol, as an antituberculosis drug is predominantly bacteriostatic, and is administered during the intensive phase of TB in an attempt to prevent further drug resistance (see Table 6.1 for further information on metabolism). Various data have suggested an interaction with aluminum-magnesium antacids, leading to a reduction in plasma ethambutol concentrations [59].

6.5.6

Ethionamide

Ethionamide is thought to be metabolized by the cytochrome P450 enzymes, and may potentially have interactions with inducers or inhibitors of this system. However, there is a paucity of data on the pharmacokinetics of this drug.

6.5.7

Fluoroquinolones

Ciprofloxacin, ofloxacin, gatifloxacin, and moxifloxacin, as fluoroquinolones, are used to treat TB via their inhibitory effect on DNA gyrase. As a class, the fluoroquinolones are not significantly affected by coadministration with food [60]. One known adverse effect of fluoroquinolones treatment is that of dysglycemia; this is especially the case when gatifloxacin is administered to patients receiving concomitant treatment for diabetes, to elderly patients, and to those who are renally

impaired [61]. The most common drug interactions with fluoroquinolones in TB therapy include malabsorption interactions associated with multivalent cations, and cytochrome P450 interactions with ciprofloxacin [62, 63]. The combination of pyrazinamide and ofloxacin appears to cause increased rates of asymptomatic hepatitis and gastrointestinal intolerance [64, 65], while combined ofloxacin and cycloserine may lead to an increased incidence of central nervous system (CNS) - mediated effects, possibly due to altered γ -aminobutyric acid (GABA) binding [66]. Modest, but potentially important, drug-drug interactions affecting the concentrations of gatifloxacin and rifampin have been reported [67]. Importantly, the Rv2686c-Rv2687c-Rv2688c genes of *M. tuberculosis* encode an ABC transporter responsible for fluoroquinolone efflux [68]; this efflux was shown to lead to a reduced accumulation of fluoroquinolone by its active removal, thereby potentially contributing to fluoroquinolone resistance in *M. tuberculosis*.

6.5.8

Streptomycin/Amikacin/Kanamycin/Capreomycin

The aminoglycoside antibiotics consist of sugar and amino moieties. Among these, streptomycin is used to treat drug-sensitive TB, while amikacin and kanamycin are used for MDR TB. As the cytochrome P450 system neither induces nor inhibits aminoglycoside activity, interactions with potent CYP inducers (rifampin) and inhibitors (protease inhibitors) do not occur. Aminoglycosides must be administered parenterally as they are poorly absorbed via the intestine. They are also not metabolized and are excreted unchanged, predominantly in the urine. The side effects of aminoglycoside are dose-dependent, and include nephrotoxicity (potentially reversible), ototoxicity (usually irreversible), and neuromuscular blockade. Thus, the concomitant administration of aminoglycosides with diuretics, radiographic contrast, angiotensin-converting enzyme (ACE) inhibitors, nonsteroidal anti-inflammatory drugs, amphotericin, and cisplatin is usually avoided [69–71]. *Capreomycin* is a peptide antibiotic that is used extensively to treat drug-resistant TB. However, as its adverse effects include nephrotoxicity and ototoxicity, its coadministration with other nephrotoxic or ototoxic agents is not advised.

6.5.9

Terizidone/Cycloserine

Terizidone is a combination of two molecules of cycloserine. A comparison of cycloserine and terizidone showed the blood levels of terizidone to be higher at all time points than those of cycloserine, although the difference was not proportional to two molecules of cycloserine being contained in one molecule of terizidone [72]. The high concentration of terizidone in urine suggests that it may be of benefit in genitourinary TB [72]. Evidence from South Africa has indicated that terizidone causes fewer adverse effects (incidence ca. 1%) than cycloserine (ca. 11%) [73]. Terizidone is a valuable companion drug to prevent resistance to other second-line

drugs, as it does not share any cross-resistance with other active TB drugs. Pyridoxine may decrease CNS-related effects, and a dose of 150 mg should be prescribed to all patients receiving terizidone or cycloserine. Terizidone should be avoided in patients with a history of epilepsy, alcoholism, and mental illness (especially depression) [73].

6.5.10

Linezolid

Linezolid does not induce cytochrome P450, and is not metabolized by this process. This is important given the possibility of its use in patients concurrently taking antiretrovirals [74, 75]. Linezolid is associated with mitochondrial toxicity, and as a result causes side effects such as peripheral neuropathy and lactic acidosis [76]. It is also known to cause reversible myelosuppression, thrombocytopenia and anemia in some patients [77]. The oral absorption (by AUC) of linezolid is unaffected by the presence of food in the intestine [78]. Drug interactions based on MAO inhibition are limited to increases in blood pressure with coadministered adrenergic agents, and are unlikely to be of any significant magnitude [79].

6.5.11

Co-Amoxyclav

The early bactericidal activity of amoxicillin/clavulanate is comparable to that reported for antituberculous agents other than isoniazid [80]. However, it has been reported unlikely that the combination of amoxicillin/clavulanic acid would have an important role in the treatment of tuberculosis, with the exception of those patients with MDR TB who otherwise are “therapeutically destitute” [81].

6.5.12

PAS

Para-aminosalicylic acid is metabolized to acetyl-PAS [82], and both compounds are excreted renally; consequently, PAS should be avoided in renal failure. The gastrointestinal toxicity of PAS also limits its use, especially as other anti-TB drugs are less likely to cause gastrointestinal side effects. Currently, PAS is formulated as granules and taken with food [83].

6.5.13

Clarithromycin

Clarithromycin is a macrolide antibiotic. Potent inhibitors of CYP3A may alter the metabolism of clarithromycin and its metabolites, while clarithromycin itself can increase the steady-state concentrations of drugs that depend primarily upon CYP3A metabolism [84].

6.6

Treatment of HIV Infection

6.6.1

Fusion Inhibitors

Enfuvirtide is a recently registered antiretroviral that inhibits HIV fusion to CD4 cells. It is not a substrate for CYP isoenzymes and neither inhibits nor induces CYP3A; thus, no significant interactions with rifamycins exist [100–102].

6.6.2

Nucleotide/Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

The NRTIs are predominantly excreted via the renal system (tubular secretion), and interactions based upon CYP are infrequent [17]. However, drugs influencing renal clearance or intracellular phosphorylation may interact with the NRTI. Significant pharmacokinetic interactions have been demonstrated when zidovudine is prescribed with probenecid, naproxen, and fluconazole [103].

Tenofovir is not a substrate, inducer or inhibitor of human cytochrome P450 enzymes (see Table 6.2). Tenofovir and rifampin may be used without dosage adjustment for the treatment of TB in HIV-infected patients [104]. However, patients with renal impairment (especially if receiving streptomycin) should be closely monitored. Tenofovir has no clinically significant drug interactions, with the exception of didanosine and atazanavir, which will require dosage modifications to be made [105]. HIV patients coinfecting with hepatitis B virus/hepatitis C virus (HBV/HCV) are likely to be treated with tenofovir and lamivudine or emtricitabine.

6.6.3

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs)

The NNRTIs and PIs are extensively metabolized by the cytochrome P450 and P-glycoprotein systems (summarized in Table 6.2). When drugs metabolized by the same pathways are administered concomitantly, pharmacokinetic drug interactions commonly result. Furthermore, ritonavir is both an inhibitor and substrate of the drug transporter P-glycoprotein [2], thus increasing the potential for drug interactions.

6.6.3.1 Oral Bioavailability of Delavirdine and PIs

The absorption of delavirdine and some PIs is affected by gastric pH and/or simultaneous food intake; typically, a reduction in gastric acidity (pH > 3) decreases the absorption of delavirdine. Indinavir is extensively (80%) absorbed from an empty stomach, and its bioavailability is decreased when administered with a fatty meal [106]. The addition of ritonavir to indinavir increases bioavailability, regardless of the stomach content [107]. The absorption of atazanavir (another PI) is dependent on gastrointestinal pH [94]. The concomitant administration of didanosine 200 mg

Table 6.2 Pharmacokinetics of antiretroviral drugs.

Drug name	CYP		P-glycoprotein		Drug metabolism (other than CYP)	Percentage (%) protein binding, principal protein bound to
	Substrate	Inducer	Inhibitor	Substrate	Inducer	Inhibitor
Nucleoside reverse transcriptase inhibitors (NRTIs)						
Zidovudine					Glucuronidation,	34–38%
Stavudine					5–10% metabolized to inactive <i>trans</i> -sulfoxide metabolite	5% <36%
Lamivudine						
Didanosine						
Zalcitabine					Alcohol dehydrogenase, glucuronosyltransferase	5% 5% 50%
Abacavir						
Nucleotide reverse transcriptase inhibitors (NRTIs)						
Tenofovir						
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)						
Efavirenz [92]	3A4, 2B6	3A4	2C9/19, 3A4	Yes		>99% albumin
Nevirapine [92]	3A4, 2B6	3A4, 2B6		Yes		60% albumin
Delavirdine [92]	3A4, 2D6, 2C9/19		3A4, 2C9, 2C19, 2D6	Yes		98% albumin
Protease inhibitors (PIs)						
Ampranavir [93]	3A4, 2D6		3A4	Yes		90% α -acid glycoprotein

(Continued)

Table 6.2 (Continued)

Drug name	CYP			P-glycoprotein		Drug metabolism (other than CYP)	Percentage (%) protein binding, principal protein bound to
	Substrate	Inducer	Inhibitor	Substrate	Inducer		
Atazanavir [94]	3A4		3A4, GT	Yes			89%, α -acid glycoprotein 86%, albumin
Darunavir [95]	3A4		3A4	Yes		Hydroxylation, hydrolysis	95% α -acid glycoprotein
Indinavir [93]	3A4, GT		3A4	Yes			60%, α -acid glycoprotein
Lopinavir [93]	3A4	GT	3A4, 2D6	Yes			98–99% α -acid glycoprotein
Nelfinavir [93]	3A4, 2C9, 2C19, 2D6	GT	3A4	Yes			glycoprotein
Ritonavir [93]	3A4, 2D6	GT, 1A2, 3A, 2C9	3A4, 2D6	Yes		Yes	>98%, α -acid glycoprotein
Saquinavir [93, 96]	3A4		3A4	Yes			98–99%, α -acid glycoprotein
Tipranavir [97]	3A4	3A4		Yes	Yes	Glucuronidation	98%, α -acid glycoprotein
Fusion inhibitors Enfuvirtide [98–100]						NADPH hydrolysis	> 99%, α -acid glycoprotein
							92%, albumin

Abbreviations: CYP: cytochrome p450 isoenzyme; GT: glucuronyl transferase; NADPH: nicotinamide adenine dinucleotide phosphate.

with atazanavir 400 mg on an empty stomach reduces atazanavir absorption by 90% (the didanosine absorption remains unchanged) [108]. The systemic absorption of darunavir is increased by 30% when taken with a meal, but acid-reducing agents have no adverse effect on bioavailability [109].

6.6.3.2 CYP Interactions in PIs

In the plasma, *darunavir* is approximately 95% bound to proteins (especially α 1-acid glycoprotein), and is extensively and almost exclusively metabolized by CYP3A4. The coadministration of darunavir with small doses of ritonavir results in an increase in the bioavailability of the former, from 37% to 82% [95].

Tipranavir induces both CYP and P-glycoprotein (see Table 6.2), and although ritonavir is a P-glycoprotein inhibitor, *in vivo* data suggest that the net effect of tipranavir/ritonavir 500 mg/200 mg is P-glycoprotein induction [110]. Tipranavir may therefore potentially decrease the absorption of concomitantly administered drugs which are substrates for P-glycoprotein [97]. The potent CYP3A4 inhibitory effects of ritonavir appear to outweigh the inducing effects of tipranavir.

6.7

Treatment Issues in Coinfection

6.7.1

Shared Toxicities

Shared drug toxicities, although clinically relevant, are beyond the scope of this chapter. Rather, the reader should consult recent reviews on this subject [3, 111].

6.7.2

TB/Antiretroviral Drug Interactions

The most significant drug–drug interaction is between rifamycins and antiretrovirals. These interactions are important, as rifamycins form the backbone of current antitubercular treatment in susceptible *M. tuberculosis* infection. An understanding of these interactions is critical to appropriate management.

6.7.2.1 Rifamycins

The rifamycins induce the cytochrome P450 enzyme system in the liver and intestinal wall, leading to a decrease in the serum half-life and in the concentrations of drugs metabolized by that system. The rifamycins vary in their ability to induce the CYP450 3A4 enzymes, with rifampin being the most potent and rifabutin the least active. In the developed world, rifabutin is the preferred rifamycin when coadministered with antiretroviral therapy; however, its high cost has hitherto precluded its use in resource-limited settings where the burden of tuberculosis and HIV is highest. As a result, the most important drug–drug interactions in the developing world are between rifampin and antiretrovirals, especially the NNRTIs and the PIs.

The autoinduction of rifampin's metabolism after repeated doses also merits attention; a pharmacokinetic study of hospitalized TB patients showed a high prevalence of low rifampin levels which could, in part, be due to autoinduction, although this might also be related to other factors such as alcohol use, gender, and drug formulation [112]. Some of these results are explained in Table 6.3, which summarizes the details of cytochrome P450 substrates and inducers.

6.7.2.1.1 Rifampin + NRTI Although there is some evidence to show that reduced levels of NRTIs such as zidovudine are observed when coadministered with rifampin as a result of rifampin inducing the glucuronidation of zidovudine [113], there is no evidence of a decrease in the intracellular concentration of the active form of the drug [114]. This implies that the efficacy of the drug is likely to be unaffected and can be safely coadministered.

6.7.2.1.2 Rifampin + NNRTI

Efavirenz There is often conflicting or lack of evidence over whether dose increments are required when this drug is given concomitantly with rifampin, although it is generally considered to be an adequate choice of antiretroviral. As rifampin is a potent inducer of the cytochrome P450 system, it leads to a decrease in the plasma levels of NNRTIs. Pharmacokinetic studies have indeed reported that rifampin decreases plasma levels. In one study, efavirenz levels were decreased by 13–25%; this was a modest decrease when compared to a 40% reduction of nevirapine and 90% with delaviridine [115], and so efavirenz is preferred for coadministration with rifampin. As a result of these pharmacokinetic studies, the CDC guidelines recommend that rifampin and delaviridine should not be used together [116]. In another study, increasing the efavirenz dose from 600 mg to 800 mg increased the plasma levels close to those observed in patients receiving 600 mg daily, without rifampin [117]. As a result of these studies, some advise that the efavirenz dose should be increased from 600 mg to 800 mg when coadministered with rifampin [118]. However, further evidence shows that trough levels are not associated with a poor clinical outcome. Several studies, using either 600 mg or 800 mg, showed no association between trough levels of efavirenz and clinical outcome, which indicated that a standard dose of 600 mg might be sufficient [119]. Furthermore, an increased dose may increase the side effects, especially in patients weighing <55 kg, and also in some black and Asian patients who have greater genetic predisposition to higher plasma levels and adverse effects. This is related to a polymorphism in CYP 2B6 enzyme in these patients, which is associated with elevated efavirenz concentrations [120, 121].

Nevirapine This is the most widely used NNRTI in resource-poor settings, as it is cheaper than efavirenz and can be used in women of childbearing age as it is not known to be teratogenic. However, it does interact with the CYP450 system, both as a substrate and inducer. Studies have shown a greater reduction in plasma levels when combined with rifampin compared to efavirenz [122]. The standard dose is an initial lead-in dose of 200 mg daily for two weeks to

Table 6.3 Substrates, inhibitors and inducers of cytochrome p450 enzymes (CYP), and P-glycoprotein (PGP).

	Substrate	Inhibitor	Inducer
CYP1A2	Acetaminophen	Erythromycin	Cigarette smoke
	Caffeine	Cimetidine	Phenobarbital
	Clozapine	Ciprofloxacin	Phenytoin
	Phenacetin	Ritonavir	Rifampin
	R-Warfarin	Erythromycin	Carbamazepine
CYP2C9/10	Theophylline	Amiodarone	Rifampin
	Dapsone	SMX/TMP	Phenobarbital
	Phenytoin	Isoniazid	Phenytoin
	S-Warfarin	Metronidazole	
	Tolbutamide	Fluconazole	
CYP2C19	Benzodiazepine	Cimetidine	Rifampin
	Proton-pump inhibitors	Diazepam	Phenobarbital
		Fluoxetine	Phenytoin
		Omeprazole	
		Isoniazid	
CYP2D6	Amiodarone	Amiodarone	
	Amitriptyline	Cimetidine	
	Clomipramine	Fluoxetine	
	Clozapine	Fluphenazine	
	Codeine	Haloperidol	
	Desipramine	Quinidine	
	Fluoxetine	Ritonavir	
	Haloperidol		
	Imipramine		
	Metoprolol		
	Propanolol		
	Risperidone		
	Timolol		
	Halothane		Ethanol
	Acetaminophen (paracetamol)		Isoniazid
CYP3A4	Carbamazepine	Amiodarone	Phenobarbital
	Cyclosporine	Cimetidine	Carbamazepine
			Dexamethasone
			Phenobarbital
	Dapsone	Clarithromycin	Phenytoin
	Diltiazem	Erythromycin	Rifampin
	Erythromycin	Fluconazole	
	Lidocaine	Fluoxetine	
	Midazolam	Grapefruit juice	
		Indinavir	
	Nifedipine	Itraconazole	
	Quinidine	Ketonazole	
	Simvastatin	Ritonavir	
	Tacrolimus	Sertraline	

(Continued)

Table 6.3 (Continued)

	Substrate	Inhibitor	Inducer
PGP	Tamoxifen		
	Testosterone		
	Valproic acid		
	Verapamil		
	Quinolones	Cyclosporine	Rifampin
	Itraconazole	Tacrolimus	
	Digoxin		

Abbreviations: SMX-TMP: trimethoprim-sulfamethoxazole.

allow for autoinduction of the CYP450 system by nevirapine, followed by 200 mg every 12 h. In one study, this reduced bioavailability could be overcome by increasing the dose to 300 mg every 12 h, with no short-term increase in adverse events [123]. Nevirapine has a higher risk, compared to efavirenz, of hepatotoxicity. Despite these findings, several studies from a variety of settings including Spain, Thailand and South Africa, have shown that nevirapine can be used safely and effectively with rifampin, with no difference in virological and immunological outcomes despite the plasma levels of nevirapine being reduced by up to 42% in patients coadministered rifampin [122, 123]. A small study in Thailand showed comparable short-term outcomes in both groups of nevirapine and nevirapine + rifampin. In this study, the nevirapine levels were reduced by 17% with rifampin coadministration, although the majority of the trough plasma nevirapine levels were higher than the recommended trough nevirapine level. In addition, there was no increase in adverse side effects of skin rash and hepatotoxicity when nevirapine was coadministered with rifampin [123]. The largest of these studies, conducted in South Africa, showed good virological outcomes with 80% of patients in the nevirapine + rifampin group being virologically suppressed at 18 months [122]. However, the study also showed there to be a higher probability of virological failure in the first two years of therapy in this group compared to patients receiving an efavirenz-based regimen. A possible reason for these contradictory findings was suggested as being due to a drug interaction between rifampin and the lead-in dosing phase of nevirapine which could, in theory, lead to a further induction by nevirapine of a system already induced by rifampin. In general, these studies have demonstrated good clinical outcomes despite a reduced bioavailability of nevirapine by rifampin coadministration; this suggests that it may be unnecessary to increase the standard nevirapine dose.

6.7.2.1.3 Rifampin + Protease Inhibitors The induction of the CYP450 system by rifampin results in markedly decreased levels of fos-amprenavir, atazanavir, indinavir, nelfinavir, saquinavir and lopinavir, ranging from 82% to 95% [43, 116] (see Table 6.3). As a result it is not recommended that rifampin is used with any of these drugs. Ritonavir, on the other hand, can be given with rifampin, either alone or in combination with another PI such as lopinavir. As ritonavir is an inhibitor of the

CYP450 system it counteracts the effect of rifampin; this is of utility when the drug is combined with lopinavir or saquinavir [124].

6.7.2.1.4 Rifabutin + NNRTI In a study of healthy volunteers, rifabutin 300 mg daily appeared to have little or no effect on efavirenz concentrations, but the rifabutin concentrations were reduced by efavirenz. As a result, the guidelines suggest increasing the rifabutin dose to 450 mg or 600 mg to compensate for this interaction, with the efavirenz dose unchanged [125]. There does not appear to be any significant interaction between rifabutin and nevirapine, and the available data suggest that the two drugs can be safely coadministered, without any need to change the dose of either drug [126].

6.7.2.1.5 Rifabutin + NRTI There does not appear to be any significant interaction between NRTI and rifabutin.

6.7.2.1.6 Rifabutin + Protease Inhibitors The coadministration of rifabutin and PIs can lead to a reduction in PI exposure, as well as a significantly increased rifabutin concentration with an associated risk of uveitis due to toxicity [127]. Current guidelines therefore advise a reduction in the rifabutin dose by up to 75% when coadministered with a PI [128].

6.8

Drug–Disease Interactions

There is conflicting evidence regarding the effect of HIV infection on the absorption of antitubercular drugs, due to the virus itself, to HIV enteropathy, or to opportunistic infections affecting the gastrointestinal tract. A study conducted in South Africa showed 39% and 27% reductions in rifampin and ethambutol, respectively, in patients with HIV infection, none of whom had diarrhea [112]. However, another study showed no evidence that infection with HIV reduces the plasma concentrations of antituberculosis drugs [129]. Results from a study in Nairobi showed that HIV infection and diarrhea did not affect the pharmacokinetics of TB drugs [130]. Data from a study in India showed an association between malabsorption of TB drugs and patients with advanced AIDS with and without diarrhea, with a low CD4 count and gastrointestinal disturbance increasing the likelihood of malabsorption [131]. Data are available which suggest that rifabutin is less frequently malabsorbed when compared to rifampin [132]. Fluoroquinolones such as ciprofloxacin appear to be well absorbed in the presence of HIV, regardless of the CD4 cell count [133].

6.8.1

TB Drugs in Development, and Potential Interactions

Moxifloxacin is unique among fluoroquinolones in that its bioavailability is not affected by the concurrent administration of ranitidine (a histamine H₂-receptor

antagonist), it has minimal renal elimination, and it is almost entirely removed in the feces (as sulfate and glucuronide conjugates) [134]. However, the absorption of moxifloxacin is similar to that of other fluoroquinolones, which is impaired by concomitant administration of aluminum- and magnesium-containing antacids. The administration of these agents should be staggered by an interval of 2 h before or 4 h after taking the antacid [135].

6.8.2

HIV Drugs in Development, and Potential Interactions

Maraviroc, a CCR5 receptor antagonist, is metabolized by the CYP3A4 isoenzymes. The coadministration of maraviroc with rifampin has been shown to result in a reduction in the plasma concentration of maraviroc by as much as 70% [136]. As a result, the current CDC guidelines recommend an increase in the dose of this antiretroviral to 600 mg twice daily when administered with rifampin. Maraviroc could also potentially interact with PIs and NNRTIs [43]. Indeed, in one study it was shown that the PI caused a significant increase in the plasma level of maraviroc, whereas efavirenz was shown to reduce maraviroc exposure by up to 50% [136].

Raltegravir is neither a potent inhibitor nor inducer of CYP 3A4 [137], and is predominantly metabolized by glucuronidation, specifically by the enzyme UDPGT 1A1[138].

6.8.3

Other Interactions of Note

A discussion of all potential drug–drug and drug–disease interactions in HIV-infected patients receiving treatment for TB, HIV and comorbid illnesses is beyond the scope of this chapter. However, some common comorbid diseases requiring therapy will be described at this point; these therapies include oral hypoglycemic agents, anticonvulsants, and anticoagulants. For example, beta-lactams can cause changes to the gastrointestinal flora, leading to an alteration of those drugs that are dependent on enterohepatic recirculation. An alteration in the gut flora that synthesize vitamin K, thus reducing endogenous vitamin K production, can augment the effect of warfarin-mediated elevations of the concentrations of statins (e.g., lovastatin and simvastatin), and the development of rhabdomyolysis secondary to CYP3A4 inhibition has also occurred. Fluoroquinolones have also been associated with fatalities secondary to hypoglycemia in patients receiving medication to manage diabetes mellitus [139], in addition to drug- and dose-dependent prolongations of the QTc interval.

6.8.3.1 Antituberculosis Drugs and Oral Hypoglycemic Agents

There is increasing evidence to suggest that diabetes mellitus (DM) significantly increases the risk of TB. A recent meta-analysis of studies assessing the association of TB and DM showed an increase of at least threefold in the risk of active TB in people with DM [140]. Typically, after two months, the results of sputum

microscopic examinations were more often positive in diabetic patients (18.1% versus 10.0%); after six months, 22.2% of cultured sputum specimens from diabetic patients were positive for *M. tuberculosis* (adjusted odds ratio, 7.65; $p = 0.004$). The maximum plasma concentration of rifampin was above the target concentration of 8 mg l^{-1} [33] in 6% of patients with TB who had DM, compared to 47% of patients without DM. These pharmacokinetic differences might lead to an easier acquisition of drug resistance, and might help to explain the inferior bacteriological response in diabetic patients with TB [141]. Thus, it is important not only to understand any possible interactions between drugs for these two conditions, but also to tease out any interactions that are clinically significant, especially as the prevalence of DM in TB-endemic areas is rising [142]. As the oral hypoglycemic agent gliclazide is metabolized by the CYP450 2C9 isoenzymes – which are induced by rifampin – there is a clear potential for an interaction between the two drugs. A recent case study suggested such clinical significance, with a report of an increased gliclazide requirement with rifampin coadministration in a patient with type 2 DM [143]. A subsequent pharmacokinetic study in healthy volunteers showed a statistically significant reduction in the blood glucose-lowering effect of gliclazide with rifampin coadministration, that was thought to be clinically significant [144]. The results of these studies suggested that a close monitoring of blood glucose levels, with a possible adjustment of the gliclazide dose, might be required. Reports also exist of interactions between the older sulfonylureas, chlorpropamide [145] and tolbutamide, and rifampin [146, 147]. There is also evidence that rifampin significantly reduces the blood glucose-lowering effect of glibenclamide [148], repaglinide [149], and glipizide [150]. However, rifampin has not been shown to have any significant effect on the blood glucose-lowering properties of a newer sulfonylurea drug, glimepiride [31].

6.8.3.2 Antituberculosis Agents and Prednisolone

There is some evidence favoring the use of adjunctive corticosteroids in some forms of extrapulmonary TB. A recent Cochrane review concluded that steroids should be used routinely in the management of TB meningitis in HIV-negative patients. However, whilst there is some evidence of benefit in HIV-infected patients, the results are inconclusive [151]. Some data are available supporting the use of adjunctive prednisolone in TB pericarditis [152]; notably, these results highlight the need to understand the interaction of prednisolone with antituberculosis drugs. The data have shown that the bioavailability of total and free prednisolone is reduced when coadministered with rifampin [153, 154], which suggests a need for dose adjustment when the two drugs are coadministered. One study conducted in India showed that the coadministration of isoniazid with prednisolone resulted in an increased renal clearance of isoniazid, regardless of the patients' acetylator status. Yet, the same study identified an increase in the acetylation rate of isoniazid in slow acetylators, which led to a decrease in isoniazid plasma concentrations. In spite of these findings, there was no difference in clinical outcome between those patients receiving prednisolone and those not, thus raising doubt over the clinical significance of these findings [155].

6.9

Conclusions

Dual HIV-TB therapy is associated with numerous challenges, particularly drug–drug and drug–disease interactions, shared drug toxicities, IRIS, and high pill burdens. Comorbid conditions due to HIV immune suppression often necessitate additional therapy, which further exacerbates these challenges. At present, a number of drugs used to treat HIV-TB either induce, inhibit, and/or are metabolized by CYP. In particular, rifampin (a CYP inducer) and ritonavir (a CYP inhibitor) are implicated in significant drug–drug interactions. There is a paucity of data relating to CYP metabolism and drug interactions for other drugs used to treat TB, HIV and comorbid conditions. The emergence of metabolic syndrome and drug-resistant TB will challenge the therapeutic strategies of clinicians, particularly in Africa, China, and India. Clearly, the limitations of our current knowledge of these drug interactions require further investigation.

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Keywords/Abstract

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Keywords: HIV; tuberculosis; interactions; coinfection; drug metabolism; drug–drug interactions; drug–disease interactions.

Clinical Deterioration during Antitubercular Treatment at a District Hospital in South Africa: The Importance of Drug Resistance and AIDS Defining Illnesses

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Abstract

Background: Clinical deterioration on drug therapy for tuberculosis is a common cause of hospital admission in Africa. Potential causes for clinical deterioration in settings of high HIV-1 prevalence include drug resistant *Mycobacterium tuberculosis* (*M.tb*), co-morbid illnesses, poor adherence to therapy, tuberculosis associated-immune reconstitution inflammatory syndrome (TB-IRIS) and subtherapeutic antitubercular drug levels. It is important to derive a rapid diagnostic work-up to determine the cause of clinical deterioration as well as specific management to prevent further clinical deterioration and death. We undertook this study among tuberculosis (TB) patients referred to an adult district level hospital situated in a high HIV-1 prevalence setting to determine the frequency, reasons and outcome for such clinical deterioration.

Method: A prospective observational study conducted during the first quarter of 2007. We defined clinical deterioration as clinical worsening or failure to stabilise after 14 or more days of antitubercular treatment, resulting in hospital referral. We collected data on tuberculosis diagnosis and treatment, HIV-1 status and antiretroviral treatment, and investigated reasons for clinical deterioration as well as outcome.

Results: During this period, 352 TB patients met inclusion criteria; 296 were admitted to hospital accounting for 17% of total medical admissions (n = 1755). Eighty three percent of TB patients (291/352) were known to be HIV-1 co-infected with a median CD4 count of 89cells/mm³ (IQR 38–157). Mortality among TB patients admitted to hospital was 16% (n = 48). The median duration of hospital admission was 9.5 days (IQR 4–18), longer than routine in this setting (4 days). Among patients in whom HIV-1 status was known (n = 324), 72% of TB patients (n = 232) had an additional illness to tuberculosis; new AIDS defining illnesses (n = 80) were the most frequent additional illnesses (n = 208) in HIV-1 co-infected patients (n = 291). Rifampin-resistant *M.tb* (n = 41), TB-IRIS (n = 51) and drug resistant bacterial infections (n = 12) were found in 12%, 14% and 3.4% of the 352 cases, respectively.

Interpretation: In our setting, new AIDS defining illnesses, drug resistant *M.tb* and other drug resistant bacteria are important reasons for clinical deterioration in HIV-1 co-infected patients receiving antitubercular treatment. HIV-1 co-infected patients may be at increased risk of acquiring nosocomial drug resistant pathogens because profound immune suppression results in co-morbid illnesses that require prolonged inpatient admissions. Routine infection control is essential and needs to be strengthened in our setting.

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Introduction

In 2007, of an estimated total population of 47.9 million people in South Africa, an estimated 5.7 million were infected with HIV-1 and 0.35 million died from AIDS-related illnesses [1]. In 2006, the

annual tuberculosis incidence rate was estimated to be 628 cases per 100,000 of the population per annum [2]. The two pandemics of tuberculosis and HIV-1 fuel each other. The annual incidence of tuberculosis disease doubles within the first year of HIV-1 infection [3] and may reach 30% per annum in the profoundly

immune-suppressed [4]. Likewise, tuberculosis disease synergistically accelerates the progression of HIV-1 infection to Acquired Immune Deficiency Syndrome (AIDS) by inciting viral replication in immunologically activated CD4 cells [5,6,7]. In South Africa more than half of tuberculosis (TB) patients tested for HIV-1 are seropositive [8] and very many of the 22.5 million HIV-1 seropositive people residing in Sub-Saharan Africa are co-infected with *M. tuberculosis* (*M.tb*) [9].

Tuberculosis and HIV-1 control programmes in Africa are complicated by the increasing emergence of drug resistant *M.tb*, which can be associated with a very poor outcome and thus adversely affect overall tuberculosis control programme performance. In 2006, 44 HIV-1 seropositive patients died a median of 16 days after obtaining a specimen that cultured extensively drug resistant (XDR-) *M.tb* in a rural area of Kwa-Zulu Natal [10]. Nosocomial exogenous re-infection was implicated as two-thirds of patients were recently hospitalised before the diagnosis of XDR-tuberculosis and genotyping of isolates showed that 85% of patients were infected with a genetically similar isolate, belonging to the KZN family of strains. Focusing on patients worsening or not stabilising on antitubercular treatment is valuable in sentinel assessment of drug resistant *M.tb* transmission and mortality. Patients deteriorating on antitubercular treatment constitute a clinical subgroup in which one would predict higher rates of MDR- and XDR- *M.tb*. However, these patients may also deteriorate due to other reasons. Reasons for clinical deterioration [11] and death in patients on antitubercular treatment include co-morbid conditions [12,13,14,15], exogenous re-infection or endogenous development of drug resistant *M.tb* [10,16], tuberculosis associated-immune reconstitution inflammatory syndrome (TB-IRIS) [17,18] and drug toxicities from treatment for tuberculosis, HIV-1 and/or concurrent opportunistic infections [19,20,21,22]. Other causes are an incorrect diagnosis of tuberculosis, a paradoxical tuberculosis reaction (if not receiving antiretroviral therapy), poor adherence [23], incorrect antitubercular treatment, malabsorption of antitubercular treatment [24], as well as malabsorption of antibiotic drugs and subsequently altered pharmacokinetics (particularly in HIV-1 infected individuals with gastrointestinal problems). It is important to derive a rapid diagnostic work-up to determine cause of clinical deterioration as well as specific management to prevent further clinical deterioration and death.

This study aimed to determine which of these reasons for clinical deterioration on antitubercular treatment was most significant at an exceptionally busy urban district hospital situated in an area of high HIV-1 prevalence.

Methods

Setting

A prospective observational study conducted from 9 January to 8 April 2007 at GF Jooste Hospital (GFJH). GFJH is an urban 200-bed adult (>15 years age) district hospital in Cape Town, South Africa that serves approximately 1.3 million people and receives 8,000 referrals per month [25] from 30 primary care clinics.

The national tuberculosis programme manages new tuberculosis cases with 6 months treatment (isoniazid, rifampin, pyrazinamide, and ethambutol [HRZE] for 2 months followed by HR for 4 months [2HRZE/4HR]). The retreatment regimen adds streptomycin (S) as follows 2HRZES/1HRZE/5HRE. New tuberculosis cases do not routinely have tuberculosis drug susceptibility testing (DST) performed. Retreatment cases and

patients not responding to antitubercular treatment may have DST performed.

Over 10,000 people have initiated combination antiretroviral treatment (cART) within the catchment area of GFJH (Meg Osler, Provincial Government of the Western Cape- personal communication). First-line cART in South Africa is stavudine (d4T), lamivudine (3TC) and either nevirapine or efavirenz (NVP, EFZ). EFZ is preferred in patients receiving rifampin-based antitubercular treatment. Patients with a CD4 count less than 200 cells/mm³ and/or a history of a WHO stage 4 illness are eligible to commence cART [26]. In patients diagnosed with tuberculosis, cART is deferred if there is no history of a WHO Stage 4 illness and the CD4 count is greater than 200 cells/mm³. If there is a history of a WHO Stage 4 illness and/or the CD4 count is less than 200 cells/mm³ cART is commenced 2 months after initiating antitubercular treatment. If the CD4 count is less than 50 cells/mm³ or a serious HIV-1 related illness exists, cART can be commenced two weeks after initiating antitubercular treatment [26].

Study procedures

Eligibility. Adult patients (>15 years) who received ≥ 14 days antitubercular treatment within GFJH's catchment area and were referred by a medical doctor or nurse to the Emergency Department or Infectious Diseases Unit were assessed. Prior to referral, patients were diagnosed with tuberculosis at any of the 12 tuberculosis clinics within the catchment population of GF Jooste Hospital. If they deteriorated and were referred to GF Jooste Hospital they underwent renewed/repeated diagnostic procedures to either confirm tuberculosis disease (if previously microbiologically unconfirmed) and to exclude drug resistant *M.tb*. Patients were eligible if clinical worsening or failure to stabilise on therapy was confirmed on clinical assessment by a medical doctor at GFJH.

Tuberculosis diagnosis and referral. Microbiological confirmation of tuberculosis disease was defined as a specimen that cultured *M.tb* and/or was smear positive for acid-fast bacilli and that was obtained from a patient with symptoms and signs of tuberculosis [26]. An empiric diagnosis of tuberculosis was defined as follows: antitubercular treatment was initiated when the tuberculosis specimen was both smear negative for acid fast bacilli and culture negative or pending for *M.tb* but the South African National Tuberculosis Control Programme's case definitions for smear-negative and extra-pulmonary tuberculosis were fulfilled [26]. Patients commenced antitubercular treatment at their tuberculosis clinic and if they deteriorated or did not improve on treatment they were referred to our hospital for assessment.

Data collection. Clinical data regarding tuberculosis diagnosis, antitubercular treatment, HIV-1 status and antiretroviral therapy were recorded. Reasons for clinical deterioration were determined by clinical assessment and laboratory investigations performed by medical staff (doctors) working in the Emergency Department and Infectious Diseases Unit. All TB patients admitted to hospital were assessed by a specialist physician and reviewed weekly during inpatient stay by an Infectious Diseases physician. Investigations performed were according to clinical presentation and included C-reactive protein, full blood count (also called complete blood cell count), urea and electrolytes, serological and blood culture investigations. Specimens for tuberculosis microscopy, culture and sensitivity included sputum, pleural fluid, lymph node aspirates, ascitic fluid or cerebrospinal fluid (CSF). Chest radiography and computerised tomographic scanning were performed as indicated. Investigations

also included sputum direct immuno-fluorescent antigen tests (DFAT) for *Pneumocystis jirovecii* pneumonia, CSF bacterial and fungal cultures and stool microscopy for coccidian parasites and culture for bacteria. All patients who develop drug-induced hepatitis are initially managed as inpatients according to a standardised protocol. The tuberculosis diagnosis is re-evaluated by reviewing results of all tuberculosis specimens. After clinical stabilisation and return to baseline of liver function tests, patients are carefully monitored and antitubercular drugs are sequentially rechallenged in cases of non-life threatening hepatitis and microbiologically proven tuberculosis. In severe cases (coagulopathy or encephalopathy), re-challenge of antitubercular treatment is not attempted. Instead, patients are treated with an alternative antitubercular regimen that does not involve rechallenge.

Tuberculosis diagnostics. The National Health Laboratory Services performed tuberculosis diagnostics. Ziehl-Nielsen staining was performed within 24 hours on all tuberculosis specimens, while auramine staining was performed on broth culture isolates. *M.tb* liquid culture, solid media susceptibility testing for isoniazid and rifampin, and quality assurance were performed as described elsewhere [27]. According to provincial protocol ethambutol (7.5 mcg/mL), ethionamide (20 mcg/mL), amikacin (30 mcg/mL), kanamycin (6 mcg/mL) and ofloxacin (2 mcg/mL) susceptibility testing was only performed in patients with rifampin resistance. Susceptibility testing to pyrazinamide, streptomycin and the remaining three second line drugs—terizidone, capreomycin, para-aminosalicylic acid—was not performed.

Definitions. Positive cultures for *M.tb* were categorised on the basis of drug susceptibility as: (i) susceptible to both isoniazid and rifampin; (ii) mono-resistant to rifampin; (iii) resistant to at least isoniazid and rifampin (MDR- *M.tb*); (iv) resistant to isoniazid, rifampin and either resistance to an injectable agent (amikacin or kanamycin) or ofloxacin (pre-XDR *M.tb*); and (v) resistant to isoniazid, rifampin, ofloxacin, and an injectable agent or ofloxacin (XDR *M.tb*). ‘Rifampin resistance’ was defined as any resistance to rifampin and included rifampin mono-resistant-, MDR-, XDR- and pre-XDR- *M.tb* [28].

Extended-spectrum beta lactamase (ESBL) producing bacteria were defined as bacteria having clavulanate-inhibited transferable enzymes able to hydrolyse third and fourth generation cephalosporins while methicillin resistant *Staphylococcus aureus* (MRSA) had an oxacillin minimum inhibitory concentration (MIC) ≥ 4 mg/L. TB-IRIS was defined according to a clinical case definition [29]. Adherence to antitubercular treatment was assessed by patient report, tuberculosis clinic cards (upon which daily doses taken are recorded) and/or collateral information from the health care worker at the tuberculosis clinic. No specific criteria were utilised but if no subsequent cause for deterioration was found and the patient had $<80\%$ adherence, the patient was assessed as having poor adherence. An additional illness to tuberculosis was defined as a second illness in patients where the initial tuberculosis diagnosis was confirmed by smear, culture and/or initial clinical response to antitubercular treatment where smear-negative and/or extrapulmonary tuberculosis was observed. It excluded poor adherence to antitubercular treatment, rifampin resistant *M.tb*, TB-IRIS or a paradoxical tuberculosis reaction. The use of concomitant cART distinguishes IRIS from a paradoxical TB reaction; IRIS occurs in patients receiving cART while paradoxical TB reaction occurs in the absence of cART. An alternate illness to tuberculosis was diagnosed when a clinical illness initially diagnosed as tuberculosis was not confirmed by smear or culture, there was no clinical response to antitubercular treatment and evidence of an alternate illness to explain the clinical presentation and course was found.

Outcomes. The primary outcome of the study was reason for clinical deterioration despite antitubercular treatment, with a specific focus on the proportion of rifampin resistant *M.tb*. The secondary outcome was outcome of admission.

Analysis. Data analysis was conducted using STATA-10 (Stata Corporation, College Station, Texas). Descriptive statistics were employed for basic characterization of variables. Wilcoxon rank-sum tests to compare medians, and Fisher’s exact test of probability to compare proportions were used, as appropriate, to identify associations.

The Research Ethics Committee of the University of Cape Town approved the study (REF: 239/2007).

Results

Characterization of cohort

Three hundred and fifty-two patients met study inclusion criteria during the 3-month period. At initial tuberculosis diagnosis, 163 (46%) had pulmonary tuberculosis, 67 (19%) had extra-pulmonary tuberculosis and 122 (35%) had combined pulmonary and extrapulmonary tuberculosis. The median duration from initiation of antitubercular treatment to clinical presentation was 92.5 days (interquartile range, IQR 43–149). Eighty-four percent of TB patients ($n = 296$) required hospital admission for a median duration of 9.5 days (IQR 4–18). Admissions for patients deteriorating on antitubercular treatment accounted for 17% medical admissions (296/1,755) during the three-month study period.

Eighty-three percent of TB patients ($n = 291$) were HIV-1 seropositive and 9% ($n = 33$) were HIV-1 seronegative (Figure 1). In 8% ($n = 28$), HIV-1 testing was not performed prior to referral or during investigation at GFJH; reasons included: not offered voluntary counselling and testing (VCT), VCT declined or too sick to obtain informed consent. Baseline features of TB patients stratified by HIV-1 status are shown in Table 1. HIV-1 seropositive TB patients were more frequently female, and less likely to have a past history of tuberculosis or microbiological confirmation ($p \leq 0.01$). The median CD4 count among HIV-1 seropositive TB patients was 89 cells/mm³ ($n = 270$). Of 255 patients who met the South African Department of Health criteria to receive cART [26], 122 (48%) were receiving this treatment.

Final diagnoses

An additional illness to tuberculosis was detected in 72% of TB patients ($n = 232$) with known HIV-1 seropositive and HIV-1 seronegative status combined ($n = 324$) (Figure 1). Rifampin-resistant *M.tb* ($n = 41$), TB-IRIS ($n = 51$) and other drug resistant bacterial infections ($n = 12$) were diagnosed. Twenty-four TB patients had poor adherence to antitubercular treatment and fifteen patients had an alternate illness to tuberculosis.

Additional illness to tuberculosis. Additional illnesses to tuberculosis differed according to HIV-1 status (Figure 1). In the HIV-1 seropositive group (Table 2), new AIDS defining illnesses ($n = 80$), bacterial infection ($n = 53$), gastroenteritis ($n = 37$) and drug toxicity to cART, antitubercular, antibiotic or antineoplastic medications ($n = 35$) were most frequent. In the HIV-1 seronegative group, complications of tuberculosis, or its therapy ($n = 7$), and bacterial infection ($n = 6$) were most common.

Antitubercular drug resistance. Rifampin resistance *M.tb* was found in 41 TB patients. Eight had rifampin mono-resistant *M.tb*, 24 had MDR- *M.tb*, four had pre-XDR- *M.tb* and five had XDR- *M.tb*. Extended sensitivity testing was performed on only 15 (37%) of the 41 rifampin resistant tuberculosis cases; one third (5/15) of these patients had XDR- *M.tb*, while a further 27% (4/15) had pre-XDR- *M.tb*.

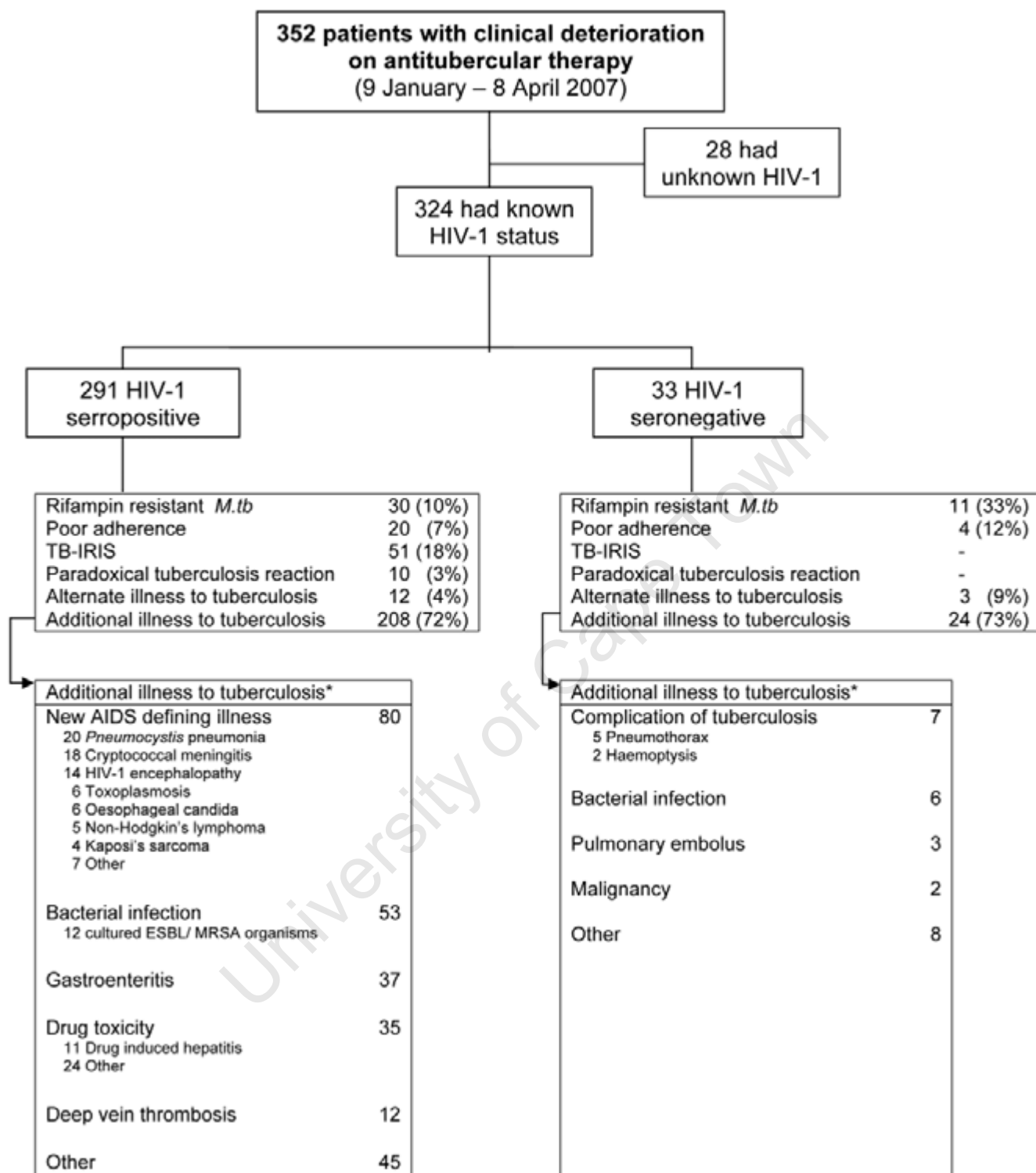


Figure 1. * Many patients had >1 cause for clinical deterioration, particularly additional illnesses. HIV-1 = human immunodeficiency virus, *M.tb* = *Mycobacterium tuberculosis*, TB-IRIS = tuberculosis associated-immune reconstitution inflammatory syndrome, Paradoxical tuberculosis reaction = initial clinical improvement with subsequent recurrence of tuberculosis clinical features but no evidence of drug resistant tuberculosis/or any other illness and patient not receiving antiretroviral therapy, MRSA = methicillin resistant *Staphylococcus Aureus*, ESBL = extended spectrum beta lactamase producing organism.

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Table 1. Characteristics of patients deteriorating on antitubercular treatment (N=324) by HIV-1 status.

	HIV-1 +ve (n = 291)	HIV-1 -ve (n = 33)	P-value
Demographics			
Male – N (%)	138 (47.4)	24 (72.7)	0.006
Median age – years (range)	34 (16–86)	37 (19–68)	NS ¹
Basis of tuberculosis diagnosis			
Smear +ve – N (%)	106 (36.4)	22 (66.7)	0.001
Smear –ve/Culture +ve – N (%)	28 (9.6)	1 (3.0)	NS
Empiric diagnosis – N (%) ²	157 (54.0)	10 (30.3)	0.01
Previous tuberculosis			
–n (%)	101 (34.7)	19 (57.6)	0.01
Admitted			
–n (%)	238 (81.8)	31 (94.0)	NS
Median duration – days (IQR ³)	10 (4–18)	11 (5–22)	NS
Outcome			
Died as inpatient – N (%)	43 (14.8)	5 (15.2)	NS
Illness contributory to death			
Bacterial illness	12	1	
Enteric illness	8	-	
<i>Pneumocystis jiroveci</i> pneumonia	6	-	
Venous thromboembolism	5	2	
Drug side effects	3	-	
Rifampin resistant-tuberculosis ⁴	3	-	
Kaposi sarcoma	2	-	
Cryptococcal meningitis	2	-	
Neurological TB-IRIS	2	-	
Chronic renal failure	-	1	
Acute coronary syndrome	-	1	

¹NS: not significant, p-value significant at $p \leq 0.05$.

²i.e. No microbiological proof at commencement of tuberculosis treatment.

³Interquartile range.

⁴Rifampin resistant-tuberculosis = *Mycobacterium tuberculosis*, with resistance to at least rifampin, cultured from a patient with clinical deterioration of symptoms attributable to progressive tuberculosis disease.

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Figure 2 depicts TB culture results at initial TB diagnosis (left figure) and at subsequent deterioration (right figure). At initial tuberculosis diagnosis specimens were sent for tuberculosis culture in 237/352 (67%) patients, with 131 TB patients having a positive culture (Figure 2). Of 131 patients that cultured *M.tb* from specimens at initial tuberculosis diagnosis, 46 (35%) did not have drug susceptibility testing performed. Of the 85 TB patients who did have tuberculosis drug sensitivity analysis at diagnosis, 12 (14%) cultured rifampin resistant *M.tb* and 73 patients (86%) cultured rifampin sensitive *M.tb*.

At clinical deterioration specimens were sent for tuberculosis culture in 234/352 (66%) patients, with 69 patients culturing *M.tb*. Of these 69 patients, 4 (6%) did not have drug susceptibility testing performed. Of 65 patients who did have antitubercular drug sensitivity testing at clinical deterioration, 35 patients (54%) cultured rifampin resistant *M.tb* and 30 patients (46%) cultured rifampin sensitive *M.tb*.

Thus, at clinical deterioration, 29 new cases of rifampin resistant tuberculosis were diagnosed. These 29 cases had the following

Table 2. Organisms cultured and sites from which they were obtained*.

Organisms cultured	n	Site of cultured organisms	n
<i>Escheriae. coli</i>	17	Blood	13
<i>Klebsiella spp</i>	5	Urine	10
<i>Staphylococcus aureus</i>	8	Soft tissue abscess	9
<i>Proteus</i>	3	Sputum	3
<i>Acinetobacter</i>	3	Ascitic fluid	2
<i>Enterococcus</i>	2	Faeces	1
<i>Enterobacter</i>	1		
<i>Haemophilus influenza</i>	1		
<i>Streptococcus anginosus</i>	1		
<i>Streptococcus pneumonia</i>	1		
<i>Pseudomonas</i>	1		
<i>Salmonella type C</i>	1		

*44 organisms were cultured from 38 sites from 35 patients: 3 patients cultured MRSA from ≥ 2 sites, 5 patients cultured 2 clinically significant bacteria from a single site.

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tuberculosis results at initial diagnosis: 6 *M.tb* sensitive to rifampin and isoniazid, 4 *M.tb* no sensitivities requested, and 3 no mycobacteria cultured. Sixteen of these 29 new cases of rifampin resistant tuberculosis had no tuberculosis culture sent at initial tuberculosis diagnosis.

Of 73 patients who had rifampin sensitive *M.tb* at initial tuberculosis diagnosis, eight (11%) cultured rifampin resistant *M.tb* at clinical deterioration. Of these eight, 2 patients cultured rifampin-mono-resistant *M.tb*, 5 MDR- *M.tb* and 1 XDR- *M.tb*, 12 to 419 days following the start of antitubercular treatment. Thirty seven percent of patients with rifampin resistant *M.tb* (15/41) are known to have died within six months of initial assessment at GFJH. The median duration from assessment to death was 28 days (IQR 11–72).

Forty-one patients who at initial TB diagnosis were culture negative (n = 11) or no culture was sent (n = 30), subsequently cultured *M.tb* at deterioration. In 11% (39/352) of cases, no tuberculosis cultures were sent at initial tuberculosis diagnosis and at clinical deterioration.

Extended spectrum beta lactamase producing organisms/methicillin resistant *Staphylococcus aureus*. At clinical deterioration, 214/352 (61%) TB patients had specimens sent for bacterial culture (128 of these TB patients had blood cultures performed). Of 214 TB patients that had ≥ 1 specimens sent for bacterial culture, 35 (16%) cultured clinically significant organisms. Cultured organisms and sites from which these specimens were obtained are detailed in table 2.

Of 53 HIV-1 seropositive TB patients treated empirically for bacterial infections (Figure 1), 32 had confirmatory cultures. Twelve of these TB patients cultured either extended spectrum beta lactamase (ESBL) producing organisms or methicillin resistant *Staph. aureus* (MRSA) (Table 3). The median CD4 count of these 12 TB patients was 57 cells/mm³ (IQR 21–80). Of nine TB patients culturing ESBL organisms, five had intermediate or high level resistance to amikacin while seven had high level resistance to ciprofloxacin. All were sensitive to imipenem, meropenem or piperacillin-tazobactam. Four TB patients had a septic illness due to MRSA, all were sensitive to vancomycin. One TB patient cultured both ESBL and MRSA organisms. Antibiotics

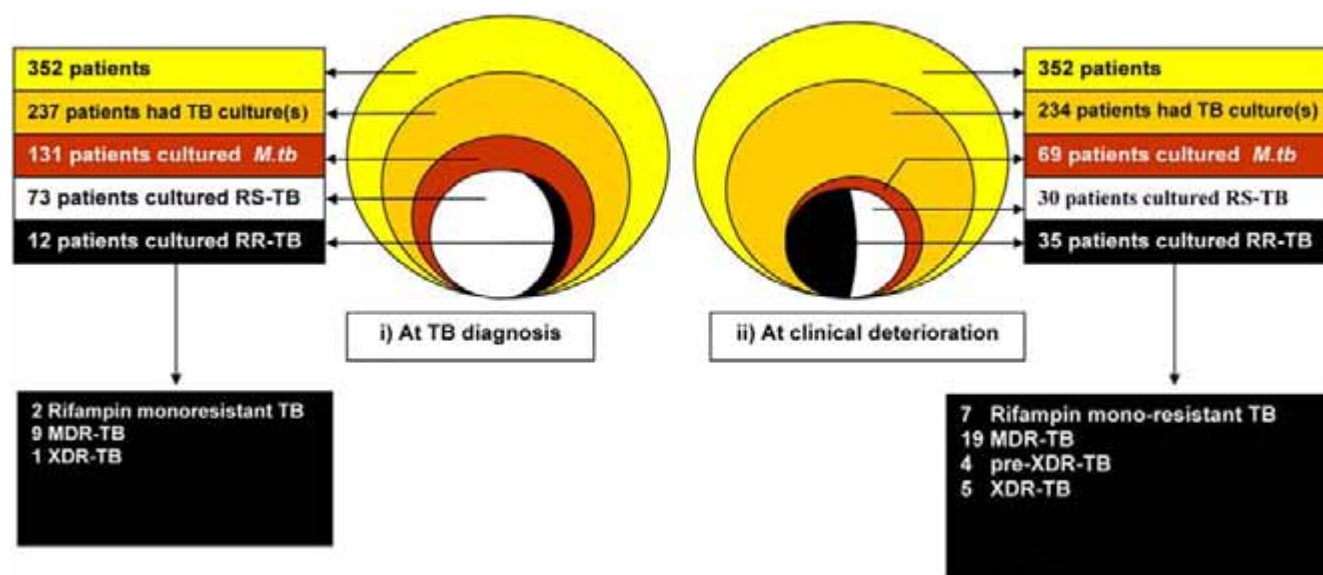


Figure 2. TB = Tuberculosis, RS-TB = rifampin sensitive *M. tuberculosis*, RR-TB = rifampin resistant *M. tuberculosis*. 6 of 12 patients who had rifampin resistant *M. tuberculosis* at initial tuberculosis diagnosis, re-cultured rifampin resistant *M. tuberculosis* at clinical deterioration, i.e. 29 new cases of rifampin resistant *M. tuberculosis* were diagnosed at clinical deterioration, 8 of 73 patients who had rifampin sensitive *M. tuberculosis* at initial tuberculosis diagnosis cultured rifampin resistant *M. tuberculosis* at clinical deterioration.
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administered to these 12 TB patients are shown in Table 3. Of these 13 drug-resistant isolates, nine (69%) were drug resistant bacteria cultured ≥ 48 hours after admission at GFJH (range 2–21 days) while four (31%) were cultured on the day of admission. Six of the twelve (50%) TB patients were admitted to a hospital in the month preceding this GFJH admission for a median duration of 10 days (IQR 8–26). Nine TB patients (75%) with these infections died with the median time from obtaining the specimen to death being 10.5 days (IQR 6.3–14.5).

TB-IRIS. Fifty one (18%) of the 291 HIV-1 seropositive TB patients were diagnosed with TB-IRIS. Their median CD4 count nadir was 65 cells/mm³ (IQR 33–113). The median interval between initiation of tuberculosis therapy and initiation of cART was 69 days (IQR 35–94), and the median interval between cART initiation and onset of TB-IRIS symptoms was 14 days (IQR 7–24).

Alternate illness to tuberculosis. An alternate illness to tuberculosis was found in 9% of patients (16/181) who started empiric antitubercular treatment. In the HIV-1 infected group ($n = 12$) alternate illnesses were predominantly new AIDS defining illnesses including Kaposi's sarcoma ($n = 2$), *Pneumocystis jirovecii* pneumonia ($n = 2$) and non-Hodgkin's lymphoma ($n = 2$).

Inpatient mortality

Inpatient mortality did not differ significantly according to HIV-1 status ($p = 0.566$, Table 1). Inpatient HIV-1 seropositive deaths ($N = 43$) were mainly due to bacterial infections ($n = 12$), new AIDS defining illnesses ($n = 10$), enteric illnesses ($n = 8$), and pulmonary embolism ($n = 5$).

An estimated 3500 patients start antitubercular treatment every three months at public sector primary care tuberculosis clinics within our catchment area (Judy Caldwell, Western Cape Tuberculosis Control Programme- personal communication). Private sector antitubercular treatment is not provided in our catchment area. During the 3-month study period 352 patients (10%, 95% confidence interval: 9–11%) deteriorated despite antitubercular treatment and required referral to our facility.

Discussion

We undertook this study to investigate which reasons for clinical deterioration on antitubercular treatment were most significant in a resource-limited setting with a high prevalence of tuberculosis HIV-1 co-infection in Cape Town, South Africa. Focusing on this particular patient group may improve their outcomes and contribute to a rational use of limited resources especially as in our setting they account for 10% of patients started on antitubercular treatment.

We found that drug resistant *M.tb* and drug resistant bacterial infections were important reasons for clinical deterioration and death. Additional illnesses to tuberculosis accounted for most referrals, especially bacterial infections and new AIDS-defining illnesses. Our findings are best explained in the context of rigorous admission criteria due to bed pressures at the 200-bed hospital; only 7.5% of adult patients referred to the emergency department are admitted to medical wards. Admission is prioritised for life-threatening illnesses requiring intravenous fluids or intravenous antibiotics (such as antibacterial or antifungal agents), drug resistant *M.tb* requiring daily inpatient intramuscular amikacin or kanamycin injections (while awaiting a bed at the nearby MDR-tuberculosis hospital) or patients requiring monitored supervision of antitubercular treatment because of severe disease. It is inevitable that a larger contingent of patients with clinical deterioration not meeting such strict admission criteria is not referred to hospital for assessment. A prospective study is needed to determine whether such a group exists, and if so whether the causes for clinical deterioration differ.

Nearly one-sixth of our patients cultured either drug resistant *M.tb* or other drug resistant bacterial infections. This has two important implications. Firstly we have identified a clinical subgroup of patients, namely patients deteriorating on antitubercular treatment, in whom the incidence of rifampin resistant tuberculosis (8.2%, 95% confidence interval: 5–11%) is high compared to the 2.5% of all tuberculosis cases reported to have MDR-tuberculosis in South Africa [30]. Secondly, tuberculosis

Table 3. Profile of patients with ESBL and MRSA organisms.

Case	Organism cultured	Site of specimen	Susceptibility to amikacin (A)/ ciprofloxacin(C)/ vancomycin (V)			Antibiotic received	CD4* (cells/mm ³)	cART	Outcome of admission	Duration from obtaining specimen to death (days)	Specimen obtained >48 hrs after admission	Admitted to hospital in previous 30 days	Duration of previous hospital admission (days)
			A	C	V								
1	<i>Klebsiella spp.</i> (ESBL)	Blood	R	R	-	Ceftriaxone+ciprofloxacin	4	Yes	Died	5	Yes	No	-
2	<i>Klebsiella spp.</i> (ESBL)	Blood	I	R	-	-	89	No	Died	22	Yes	Yes	5
3	<i>Klebsiella spp.</i> (ESBL)	Blood	I	R	-	Ceftriaxone	77	Yes	Died	1	Yes	Yes	72
	MRSA	Pus swab ×2	-	-	S					13	No		
4	<i>E. coli</i> (ESBL)	Blood	I	R	-	Amikacin	20	Yes	Died	61	Yes	Yes	11
5	<i>E. coli</i> (ESBL)	Blood	S	R	-	Amikacin	51	No	Died	6	Yes	Yes	9
6	<i>E. coli</i> (ESBL)	Blood	S	R	-	Amikacin	76	No	Discharged alive	-	Yes	No	-
7	<i>E. coli</i> (ESBL)	Midstream urine	S	R	-	Amikacin	unknown	Yes	Discharged alive	-	Yes	No	-
8	<i>E. coli</i> (ESBL)	Blood	R	S	-	-	167	Yes	Died	10	No	Yes	30
9	<i>E. coli</i> (ESBL)	Blood	S	S	-	Amikacin	21	No	Died	11	No	No	-
10	MRSA	Pus swab ×2	-	-	S	Cloxacillin	38	No	Died	10	Yes	No	-
11	MRSA	Blood	-	-	S	Vancomycin	166	No	Died	8	Yes	No	-
12	MRSA	Pus swab ×2	-	-	S	Clindamycin	62	Yes	Discharged alive	-	No	Yes	7
Median (IQR)							CD4 = 57 (21–80)			10 (8–14)			
Percentage								50%	Died 75%		Yes, 69%	Yes, 54%	

Klebsiella spp. (ESBL) = *Klebsiella spp.* demonstrating extended spectrum beta-lactamase activity, sensitive to Imipenem, Meropenem or Piperacillin-tazobactam.

E. coli (ESBL) = *Escherichia coli* demonstrating extended spectrum beta-lactamase activity, sensitive to Imipenem, Meropenem or Piperacillin-tazobactam.

MRSA = Methicillin resistant *Staphylococcus aureus*, resistant to cloxacillin.

Susceptibility: R = Resistant, I = Intermediate resistance, S = sensitive.

cART = combination antiretroviral therapy.

* = all 12 patients were HIV-1-infected.

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HIV-1 co-infected patients in a hospital setting appear prone to acquire drug resistant bacteria such as ESBL and MRSA organisms. Studies need to be conducted at other health facilities to determine whether similar causes for clinical deterioration exist.

Nosocomial acquisition of XDR- *M.tb* among HIV-1 infected patients with a very poor outcome has recently been described in South Africa [10]; the contribution to death by co-morbid HIV-1 associated illnesses, however, was not discussed in that report. Our study suggests co-morbidities may play a role in the death of such patients.

The evolution from isoniazid and rifampin sensitive *M.tb* to rifampin mono-resistant-, MDR-, preXDR- *M.tb* and XDR- *M.tb* in eight patients may be due to initial mixed *M.tb* strain infection [31], exogenous re-infection with rifampin resistant *M.tb* [16] or the rapid development of *M.tb* drug resistance mutations despite a multidrug-regimen i.e. amplified drug resistance. Nosocomial re-infection of HIV-1 seropositive patients with MDR- and XDR- *M.tb* has been documented in both resource rich and resource constrained settings [10,16]. The possibility that drug resistance evolved rapidly in drug susceptible cases receiving optimal treatment appears less likely based on clinical history and collateral information regarding adherence obtained from tuberculosis clinics and relatives. The decline in proportion of patients culturing *M.tb* may reflect either efficacious antitubercular treatment, inability of the patient to expectorate sputa for culture, or difficulty obtaining a non-pulmonary specimen for culture.

Nosocomial acquisition of drug resistant bacteria is also suggested by the temporal association between i) the timing of specimens that cultured ESBL and MRSA organisms and ii) the duration of current and previous hospital admissions. The unavailability of appropriate antimicrobial agents likely contributed to the high mortality rate in these 12 HIV-1 seropositive TB patients. At the time of the study, the standard regimen for suspected nosocomial sepsis was amikacin and ceftriaxone in the absence of renal impairment, and ciprofloxacin and ceftriaxone if present. Vancomycin was available to treat MRSA. Because of our study findings, ertapenem is now available.

The incidence of additional illnesses such as new AIDS defining illnesses, bacterial infections and gastroenteritis is indicative of the profound immune suppression in the HIV-1 infected group. Multiple opportunistic infections occur simultaneously in AIDS patients. A necropsy study of HIV-1 infected patients from Brazil reported more than one post-mortem diagnosis in 52% of the patients, and 48% had at least one AIDS-related disease not suspected clinically [32]. These researchers recommended aggressive investigation for infections and cancers in sick patients with AIDS, particularly in those not responding to initial antimicrobial therapy [32]. Bacterial infections and enteric illnesses were found in 26% and 18% of HIV-1 infected patients, respectively, in our study. Current provincial government protocols recommend starting co-trimoxazole prophylaxis in all HIV-1 positive patients one month after initiating antitubercular treatment in order to differentiate between side effects from antitubercular treatment and co-trimoxazole [26,33]. This would likely reduce bacterial infections. Adherence to this recommendation was not assessed and needs to be determined in future studies. Although only 48% of patients eligible for cART were receiving cART at assessment

for deterioration, it is likely that some patients subsequently initiated cART.

TB-IRIS was a final diagnosis in 18% (51/291) of HIV-1 seropositive patients. This probably reflects the high incidence of disseminated tuberculosis and the relatively late initiation of cART in profoundly immune suppressed HIV-1 patients in our setting. TB-IRIS is more likely to occur in patients with a low baseline CD4 count, a short duration between initiation of antitubercular treatment and cART and disseminated tuberculosis [34,35,36].

Fifteen cases of venous thrombo-embolic disease (12 deep vein thrombosis and 3 pulmonary embolus) were observed among this cohort. HIV-1 and rifampin are postulated risk factors for venous thrombo-embolic disease [37,38].

Both HIV-1 uninfected and infected patients had prolonged admissions (9.5 days) compared to the typical duration of admission at GFJH (4 days) [25]. Longer inpatient admissions increase the risk of acquisition of nosocomial drug resistant pathogens, particularly in immune-compromised patients.

Our study's limitations relate fundamentally to its design within routine care in an exceptionally busy setting. Studies based in hospitals suffer referral bias and so the extent of the problem of clinical deterioration during antitubercular treatment cannot be precisely determined although is clearly very significant and likely to impact adversely on overall national tuberculosis programme success. The initial tuberculosis diagnosis was often defined by clinical algorithm rather than bacterial culture and, even at clinical deterioration, not all patients were sampled. Of all 352 TB patients assessed at deterioration, 182 (52%) did not culture *M.tb* at both tuberculosis diagnosis and at deterioration. Final diagnosis relied on available diagnostic modalities, better than in many parts of Africa but not state-of-the-art. Resistance to second line antitubercular agents was not always assayed: thus our estimates of pre-XDR and XDR- *M.tb* may be falsely low. 8% of patients were not tested for HIV-1 infection. Genotyping of drug resistance *M.tb* and other bacterial strains would have allowed us to better assess the likelihood of nosocomial transmission. All these factors have been considered in a clinic-based second study of this problem that is currently in progress.

As a result of these findings, basic infection control measures have been strengthened; extraction fans have been installed at high congestion areas and natural ventilation is encouraged to reduce *M.tb* transmission. N95 respirator masks are readily available to patients, relatives and health care workers to reduce aerosol transmission and infection of tuberculosis. Simple architectural modifications in the hospital are currently underway to further improve ventilation and reduce *M.tb* transmission risk.

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Author Contributions

Conceived and designed the experiments: DJP CM RJW GAM. Performed the experiments: DJP KR RJW GAM. Analyzed the data: DJP CM. Wrote the paper: DJP KR CM RJW GAM.

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Neurologic Manifestations of Paradoxical Tuberculosis-Associated Immune Reconstitution Inflammatory Syndrome: A Case Series

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Background. Paradoxical neurologic tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is a potentially life-threatening condition that occurs within 3 months after starting combination antiretroviral therapy (ART). The reports in the published literature are anecdotal, and the prevalence and outcomes of neurologic TB-IRIS are unknown.

Methods. We prospectively assessed patients with suspected TB-IRIS from June 2005 through October 2007 at our hospital in Cape Town, South Africa. We defined paradoxical TB-IRIS and paradoxical neurologic TB-IRIS with use of consensus clinical case definitions. We collected data on tuberculosis diagnosis, ART, details of TB-IRIS diagnosis, other opportunistic infections, corticosteroid use, and outcome.

Results. We reviewed 279 patients with suspected TB-IRIS, 54 (19%) of whom had suspected neurologic TB-IRIS, and 225 (81%) of whom had suspected non-neurologic TB-IRIS. Paradoxical TB-IRIS was diagnosed in 190 patients; 23 (12%) of these 190 patients had neurologic TB-IRIS (95% confidence interval, 7%–17%). Eight had meningitis, 7 had tuberculoma, 5 had both tuberculoma and meningitis, and 3 had radiculomyelopathy. Twenty (87%) of the 23 patients with neurologic TB-IRIS required hospital admission (median duration, 12 days; interquartile range, 6–24 days), and 21 (91%) received corticosteroids (median duration, 58 days; interquartile range, 29–86 days). Outcomes 6 months after the initial assessment for neurologic deterioration were as follows: 16 (70%) of the patients were alive (10 of these patients had documented full physical and mental recovery), 3 (13%) were dead, and 4 (17%) were lost to follow-up.

Conclusions. Paradoxical neurologic TB-IRIS accounts for 12% of paradoxical TB-IRIS cases. Neurologic TB-IRIS causes considerable short-term morbidity but has reasonable long-term outcomes. Further research is needed to devise optimal diagnostic and management strategies for patients with tuberculosis who experience neurologic deterioration after starting ART.

Paradoxical and unmasking tuberculosis-associated immune reconstitution inflammatory syndromes (TB-IRISs) [1] are emerging complications of combination

antiretroviral treatment (ART) in countries with high rates of tuberculosis, especially in Africa [2]. The temporal sequence of events distinguishes paradoxical TB-IRIS from unmasking TB-IRIS; antitubercular treatment precedes ART in paradoxical TB-IRIS, whereas ART precedes tuberculosis diagnosis in unmasking TB-IRIS [1]. Currently, no confirmatory diagnostic test for paradoxical TB-IRIS exists. Differential diagnoses include failure of antitubercular treatment because of antimicrobial resistance or suboptimal antitubercular drug concentrations, drug reactions, and alternative opportunistic conditions [3, 4]. Published case definitions require the exclusion of these differential diagnoses, if possible, before diagnosis of paradoxical TB-IRIS [1, 5,

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6]. Severe and life-threatening manifestations of paradoxical TB-IRIS include respiratory failure [7, 8] and neurologic involvement [9–13]. Several case reports, but no case series, of paradoxical neurologic TB-IRIS have been published [9–13].

In paradoxical neurologic TB-IRIS, inflammation in the central nervous system (CNS) may result in death or permanent neurologic disability. Adjunctive corticosteroid therapy is often used to treat neurologic TB-IRIS, despite a lack of evidence of benefit. Determining the cause of neurologic deterioration in patients with tuberculosis who are receiving ART is important; inappropriate adjunctive corticosteroid therapy for patients with immunosuppression who actually have suboptimally treated tuberculosis or other untreated opportunistic infections may be fatal. However, if treatment with corticosteroids is effective, then failure to administer them may have severe consequences. Unfortunately, access to expensive tests, such as neuroimaging and drug susceptibility testing, is poor in resource-limited settings, where most of the burden of disease exists.

In this study, we evaluated patients with suspected TB-IRIS who were referred to our hospital to determine causes for neurologic deterioration. We used a published consensus clinical case definition of paradoxical TB-IRIS for resource-limited settings [1] to identify paradoxical neurologic TB-IRIS. Here, we describe the clinical presentation, management, and outcomes of paradoxical neurologic TB-IRIS and discuss challenges of diagnosis and management in resource-limited settings.

PATIENTS AND METHODS

Study site and setting. We conducted a prospective study at GF Jooste Hospital (Cape Town, South Africa) from 1 June 2005 through 31 October 2007. GF Jooste Hospital is a secondary-level, 200-bed, public-sector hospital that serves indigent adult patients (≥ 15 years of age) who are referred from primary health care clinics in periurban communities of Cape Town. GF Jooste Hospital receives ~ 8000 referrals per month [14] from 30 primary care clinics with a catchment population of 1.3 million people.

In 2006, the reported tuberculosis case notification rate in the Western Cape was 1031 cases per 100,000 population [15], and the antenatal human immunodeficiency virus type 1 (HIV-1) seroprevalence was 15% (95% confidence interval [CI], 12%–19%) [16]. According to national protocol, patients with a new diagnosis of tuberculosis receive 6 months of treatment (isoniazid, rifampin, pyrazinamide, and ethambutol for 2 months, then isoniazid and rifampin for 4 months) [17]. The retreatment regimen adds streptomycin, as follows: 2 months of isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin, 1 month of isoniazid, rifampin, pyrazinamide, and ethambutol, and 5 months of isoniazid, rifampin, and ethambutol. New patients with neurologic tuberculosis receive pro-

longed treatment of 9 months (2 months of isoniazid, rifampin, pyrazinamide, and ethambutol, and 7 months of isoniazid and rifampin) [17]. New patients with tuberculosis do not routinely undergo drug susceptibility testing; patients receiving retreatment and patients who do not respond to antitubercular treatment may undergo drug susceptibility testing.

First-line ART in South Africa is stavudine, lamivudine, and either nevirapine or efavirenz. Efavirenz is preferred for patients who are receiving rifampin-based tuberculosis treatment. Patients with a CD4⁺ cell count < 200 cells/ μ L and/or a history of a World Health Organization stage 4 illness are eligible to commence ART [18]. ART is commenced 2 months after initiation of antitubercular treatment. If the CD4⁺ cell count is < 50 cells/ μ L or a serious HIV-1–related illness is present, ART may be commenced 2 weeks after starting antitubercular treatment [18]. To date, $> 12,000$ people have initiated ART at primary care clinics within the catchment area.

Definitions. Patients with suspected TB-IRIS were defined as patients with HIV-1 infection who received antitubercular treatment, then commenced ART, and subsequently experienced deterioration within 3 months, with symptoms or signs compatible with tuberculosis. Patients with suspected neurologic TB-IRIS were defined as having ≥ 1 new or recurrent neurologic symptom and/or sign, including headache, focal neurologic deficit, nuchal rigidity, confusion, seizures, cerebellar signs, cognitive impairment, and/or psychiatric manifestations. We adapted the consensus clinical case definition of paradoxical TB-IRIS for resource-limited settings [1]. We expanded the major neurologic criterion of this case definition (i.e., new or worsening CNS tuberculosis, including meningitis or focal neurologic deficit [e.g., caused by tuberculoma]) to include the following 3 neurologic disease categories: (1) new or worsening tuberculous meningitis; (2) new or worsening intracerebral space-occupying lesion (probable tuberculoma); and (3) new or worsening radiculomyelopathy. The consensus clinical case definition requires exclusion of alternative explanations for clinical deterioration, if possible. In a number of cases, we were unable to definitively exclude alternative explanations for clinical deterioration, but our clinical assessment was likely paradoxical neurologic TB-IRIS. We therefore included patients (1) who died despite treatment interventions, (2) who, in addition to TB-IRIS treatment, received therapeutic trimethoprim-sulfamethoxazole (320 mg/1600 mg twice daily) to treat possible cerebral toxoplasmosis (the principal differential diagnosis in cases of tuberculoma IRIS), and/or (3) in whom antitubercular treatment was intensified from 2 drugs (isoniazid and rifampin) to 4 drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) or from 4 drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) to 7 drugs (isoniazid, rifampin, pyrazinamide, and ethambutol plus amikacin, ofloxacin, and ethionamide) (in the latter case, to cover for the

possibility of drug-resistant tuberculosis). We regard all cases of paradoxical neurologic TB-IRIS as probable, because a confirmatory test for TB-IRIS is not yet available.

We defined microbiologically confirmed tuberculosis as *Mycobacterium tuberculosis* cultured or acid-fast bacilli seen in a sputum, nodal aspirate, or CNS specimen. The CNS specimens included cerebrospinal fluid and brain or paraspinal biopsy specimens. We defined microbiologically unconfirmed tuberculosis according to World Health Organization case definitions for smear-negative and extrapulmonary tuberculosis [19]. We defined multidrug-resistant (MDR) tuberculosis as *M. tuberculosis* with resistance to isoniazid and rifampin. We defined disseminated tuberculosis as tuberculosis disease at ≥ 2 non-contiguous sites or a miliary pattern visible on chest radiograph.

We defined HIV-1 encephalopathy as chronic cognitive impairment (with or without cerebral atrophy findings on computed tomography), with exclusion of opportunistic infections as the cause for neurologic deterioration. Delirium was considered if patients presented with a systemic illness and acute cognitive deterioration. We diagnosed delirium when we observed a return to baseline cognitive function on resolution of systemic illness. We defined initial clinical improvement as symptom improvement (e.g., improvement of headache) or improvement assessed by neurologic examination (e.g., focal weakness or Glasgow Coma Scale score improvement from admission). Initial clinical improvement did not necessitate complete resolution of symptoms and/or signs. We defined residual deficit as persistence of any neurologic symptom or sign. We defined complete physical and mental recovery as a return to baseline functioning, as assessed by the physician who discharged the patient from the hospital. Lastly, we defined a patient as being lost to follow-up if we were unable to trace a patient 6 months after initial assessment for neurologic deterioration. We used hospital medical notes, as well as the National Health Laboratories Service and the Provincial Government of the Western Cape's computer databases, to trace patients.

Assessment of patients with suspected TB-IRIS and data collection. We assessed patients with suspected TB-IRIS from June 2005 through October 2007, during recruitment to a randomized controlled trial of prednisone versus placebo for mild and moderate paradoxical TB-IRIS. Before the trial, clinical case definitions for TB-IRIS were prepared, circulated, and discussed with participating primary care physicians. Patients with suspected TB-IRIS who were referred to GF Jooste Hospital were prospectively assessed (regardless of severity of illness, eventual diagnosis, or inpatient or outpatient management) to exclude differential diagnoses. For example, we excluded bacterial and cryptococcal meningitis in patients with features that were suggestive of meningitis. We also sent specimens, such as sputum, cerebrospinal fluid, and nodal aspirates, for mycobacterial

culture and drug susceptibility testing, to exclude drug-resistant *M. tuberculosis*. Patients with severe paradoxical TB-IRIS manifestations, such as respiratory failure, altered level of consciousness, or new focal neurologic signs, were excluded from the randomized controlled trial and usually received prednisone.

Data collected regarding patients with suspected neurologic TB-IRIS included tuberculosis diagnosis, antiretroviral treatment, CD4⁺ cell count before and after receipt of antiretroviral treatment, details of neurologic deterioration, eventual diagnosis, clinical management, and outcome at 6 months after presentation. The Research Ethics Committee of the University of Cape Town approved this study (REC 337/2004).

RESULTS

During the 29-month study period, we assessed 279 patients with suspected TB-IRIS (figure 1). Two hundred twenty-five (81%) of 279 patients with suspected TB-IRIS had suspected non-neurologic TB-IRIS, whereas 54 (19%) had suspected neurologic TB-IRIS, because they had ≥ 1 neurologic symptom and/or sign. TB-IRIS was diagnosed more often among patients with suspected non-neurologic TB-IRIS than it was among those with suspected neurologic TB-IRIS (166 [74%] of 225 vs. 23 [43%] of 54; $P < .001$, by χ^2 test). Among the 54 patients with suspected neurologic TB-IRIS, we diagnosed paradoxical neurologic TB-IRIS in 23 patients and other illnesses in 31 patients. Therefore, of 190 patients with a diagnosis of paradoxical TB-IRIS, 23 (12%; 95% confidence interval, 7%–17%) had neurologic TB-IRIS: 8 had meningitis, 7 had tuberculoma, 5 had both tuberculoma and meningitis, and 3 had radiculomyelopathy. Among the 31 patients with illnesses other than paradoxical neurologic TB-IRIS, the most frequent diagnoses were HIV-1 encephalopathy with delirium (7 patients), cryptococcal meningitis (5 patients), and MDR tuberculosis (4 patients).

Table 1 gives the baseline characteristics and neurologic symptoms and signs for patients with a diagnosis of paradoxical neurologic TB-IRIS. The median CD4⁺ cell count was 61 cells/ μ L (interquartile range [IQR], 29–97 cells/ μ L) before ART and 293 cells/ μ L (IQR, 128–482 cells/ μ L) a median of 183 days (IQR, 161–223 days) after ART initiation. Median duration from antitubercular treatment to initiation of ART was 65 days (IQR, 41–87 days), and median duration from ART initiation to new or recurrent neurologic symptoms was 14 days (IQR, 5–29 days). The most frequent neurologic symptoms and signs were headache (16 patients; 70%), nuchal rigidity (9 patients; 39%), and seizure(s) (7 patients; 30%). Median duration from onset of neurologic symptoms to initial hospital assessment was 6 days (IQR, 3–14 days).

Table 2 summarizes details of the 23 patients with neurologic TB-IRIS. Cerebrospinal fluid analysis for 13 patients who re-

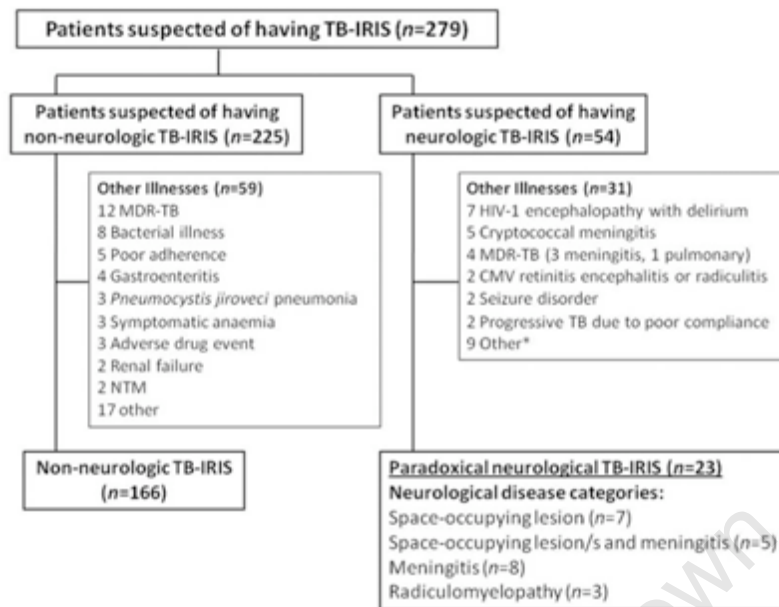


Figure 1. Diagnoses for 279 patients with suspected tuberculosis (TB)-associated immune reconstitution inflammatory syndrome (TB-IRIS) (1 June 2005–31 October 2007). Other* indicates headache cause undetermined (3 patients), motor axonopathy cause undetermined (2), cerebrovascular accident (1), nosocomial pneumonia and hypoxic ischemic brain insult (1), cauda equine lesion with undetermined cause (1), or subtherapeutic antitubercular drug level (1). CMV, cytomegalovirus; MDR-TB, multidrug-resistant TB resistant to rifampin and isoniazid; NTM, nontuberculous mycobacteria.

ceived a diagnosis of meningitis revealed the following values: median lymphocytes count, 30 cells/ μ L (IQR, 14–65 cells/ μ L); median polymorph count, 0 polymorphs/ μ L (IQR, 0–5 polymorphs/ μ L); median protein level, 1.6 g/L (IQR, 1.0–2.3 g/L); and median glucose level, 2.1 mmol/L (IQR, 1.7–2.5 mmol/L). For 21 of 23 patients, we performed computed tomography or magnetic resonance imaging of the brain or spinal cord. Space-occupying lesions were found in 13 of 21 patients who underwent brain or spinal cord imaging. Immunoglobulin G serologic analysis for *Toxoplasma* species was performed for 11 of these 13 patients, 7 of whom had positive results; however, all 7 had radiologic features of tuberculosis outside of the nervous system. We treated 7 patients with therapeutic trimethoprim-sulfamethoxazole (320 mg/1600 mg twice daily) to cover possible cerebral toxoplasmosis; 4 of these 7 patients had IgG test results positive for *Toxoplasma* species.

Six patients (patients 7, 8, 10, 11, 18, and 21) had evidence of neurologic tuberculosis before ART. The remaining patients did not have clinical features of neurologic tuberculosis before ART. At neurologic deterioration, we sent 17 patients' CNS specimens for mycobacterial culture; 16 had negative results, and 1 patient had a culture that grew *M. tuberculosis* (patient 20).

Twenty (87%) of 23 patients required hospital admission (median duration, 12 days; IQR, 6–24 days), 21 (91%) received corticosteroids (median duration of therapy, 58 days; IQR, 29–86 days), and 7 (30%) received intensified antitubercular treat-

ment. Initial clinical improvement occurred in 19 (83%) of the 23 patients; 18 of 19 patients with initial improvement received corticosteroids. Median duration from corticosteroid treatment to initial clinical improvement was 10 days (IQR, 4–22 days). Three (13%) of 23 patients died within 6 months after initial assessment for neurologic deterioration. Four (17%) of 23 patients were lost to follow-up 6 months after the initial assessment for neurologic deterioration. In these 4 patients, duration from initial assessment to loss to follow-up (presumed dead) ranged from 121 to 278 days. Known survival was thus 70% at 6 months after neurologic TB-IRIS diagnosis. We documented full physical and mental recovery in 10 (63%) of 16 patients who were alive and in care at 6 months. Six (37%) of 16 patients had residual neurologic deficit at 6 months. Below, we describe case reports of tuberculoma IRIS, tuberculous meningitis IRIS, and TB-IRIS spondylitis with radiculopathy which occurred in patients 12, 14, and 23, respectively, from tables 1 and 2.

Patient 12: paradoxical brain tuberculoma and meningitis IRIS. A 36-year-old woman with HIV infection received a diagnosis of disseminated tuberculosis. Acid-fast bacilli were seen in her sputum sample, a chest radiograph showed a miliary pattern, and abdominal ultrasound visualized splenic hypodensities, ascites, and a pericardial effusion. She commenced antitubercular treatment with isoniazid, rifampin, pyrazinamide, and ethambutol. She gained weight, her cough and sweating improved, and her fever resolved. Her CD4⁺ cell count

Table 1. Baseline characteristics, tuberculosis (TB) and human immunodeficiency virus type 1 (HIV-1) data, and details of neurologic symptoms for 23 patients seropositive for HIV-1 with paradoxical neurologic TB-associated immune reconstitution inflammatory syndrome (TB-IRIS).

Patient no.	Sex	Age, years	Previous TB	Site of TB before ART	Initial TB result (specimen site)	Duration from TB treatment to ART, weeks	Duration from ART to TB-IRIS, days	CD4 ⁺ cell count before ART, cells/ μ L	Duration from ART initiation to CD4 ⁺ cell count, days	CD4 ⁺ cell count while receiving ART, cells/ μ L	Neurologic symptoms and signs
1	M	43	No	Nodal	AFB visualized (node and sputum)	7	14	89	178	260	Headache, cognitive impairment, and left arm weakness
2	F	34	Yes	Pleural	No microbiological confirmation	8	9	25	Confusion and fever
3	M	44	No	Disseminated	<i>Mycobacterium tuberculosis</i> cultured (node)	4	19	134	224	502	Headache and focal seizures of right arm
4	M	35	Yes	Pleural	<i>M. tuberculosis</i> cultured (sputum)	12	11	139	151	423	Seizures and left hemiparesis
5	F	24	No	Disseminated	<i>M. tuberculosis</i> cultured (node)	4	3	12	23	6	Generalized tonic clonic seizures and postictal confusion
6	F	39	No	Pulmonary	No microbiological confirmation	11	28	79	306	326	Sudden-onset right arm and leg weakness with sensory loss
7	F	28	Yes	Disseminated	<i>M. tuberculosis</i> cultured (sputum)	19	2	33	Seizures and confusion
8	F	23	Yes	Disseminated	<i>M. tuberculosis</i> cultured (CSF)	9	49	254	214	1043	Headache and generalized tonic clonic seizures
9	F	37	No	Disseminated	No microbiological confirmation	5	33	17	Headache, left arm and leg seizures, and confusion
10	F	43	No	Meningitis, SOL	AFB visualized (CSF)	35	21	3	Headache, neck stiffness, confusion, and fever
11	F	30	No	Meningitis	No microbiological confirmation	13	4	95	187	423	Headache, fever, neck stiffness, and generalized tonic clonic seizures
12	F	36	No	Disseminated	AFB visualized (sputum)	19	16	50	Headache, neck stiffness, vomiting, and hysteria

13	F	30	Yes	Abdominal nodes	No microbiological confirmation	5	5	19	157	104	Headache, confusion, neck stiffness, photophobia, and vomiting
14	M	34	No	Disseminated	No microbiological confirmation	2	1	77	173	207	Headache, fever, and vomiting
15	F	33	No	Disseminated	No microbiological confirmation	10	1	61	359	539	Headache, nausea, vomiting, and night sweats
16	F	28	No	Disseminated	<i>M. tuberculosis</i> cultured (sputum)	13	8	37	110	118	Headache, fever, and neck stiffness
17	M	37	Yes	Disseminated	AFB visualized (sputum)	7	1	8	221	111	Neck stiffness, confusion, and generalized tonic clonic seizures
18	M	30	No	Meningitis	No microbiological confirmation	12	30	158	Headache, fever, vomiting, and neck stiffness
19	M	27	Yes	Disseminated	AFB visualized (sputum)	2	38	48	Headache, neck stiffness, and fever
20	M	31	No	Pulmonary	AFB visualized (sputum)	7	14	52	301	159	Headache and neck stiffness
21	F	41	No	Meningitis	No microbiological confirmation	10	30	80	Headache and worsening lower limb weakness
22	F	26	Yes	Disseminated	<i>M. tuberculosis</i> cultured (sputum)	6	7	137	Headache and lower limb weakness and incontinence of urine and feces
23	F	31	No	Disseminated	<i>M. tuberculosis</i> cultured (sputum)	34	61	99	173	703	Right leg weakness and lower back and right hip pain
All patients, median value (IQR)						9 (6–13)	14 (5–29)	61 (29–97)	183 (161–223)	293 (128–482)	...

NOTE. AFB, acid-fast bacilli; ART, antiretroviral therapy; CSF, cerebrospinal fluid; IQR, interquartile range; SOL, space-occupying lesion; TB, tuberculosis. All patients received stavudine (30 mg twice daily), lamivudine (150 mg twice daily), and efavirenz (600 mg nightly) antiretroviral treatment except patients 16, 17, and 21, who received zidovudine (300 mg twice daily), lamivudine (150 mg twice daily), and efavirenz (600 mg nightly), and patient 10, who received stavudine (30 mg twice daily), lamivudine (150 mg twice daily), and nevirapine (200 mg twice daily). Thirty-five percent of patients were male, and 65% did not have previous TB. The median patient age was 33 years (IQR, 28–37 years).

Table 2. Neurologic disease category, investigations, management, and outcome for 23 patients who are seropositive for human immunodeficiency virus type 1 (HIV-1) with paradoxical neurologic tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS).

Patient no.	Neurologic TB-IRIS category	Cerebrospinal fluid ^a				Brain or spinal imaging	TB result at neurologic deterioration
		Lymphocyte count, cells/ μ L	Polymorphonuclear cell count, cells/ μ L	Protein level, g/L	Glucose level, mmol/L		
1	SOL	CT brain: 1 SOL	AFB visualized (node)
2	SOL	0	0	1.6	3.1	CT brain: 1 SOL	Culture negative (CSF)
3	SOL	0	0	0.3	3	CT brain: multiple SOLs	No culture (CSF)
4	SOL	CT brain: 1 SOL	No specimen obtained
5	SOL	0	0	0.4	3.1	CT brain: 4 SOLs	Culture negative (CSF)
6	SOL	CT brain: 1 SOL	Culture negative (sputum)
7	SOL	3	0	0.9	2.1	MRI brain: 1 SOL	Culture negative (CSF)
8	SOL and meningitis	8	3	0.8	NP	CT brain: 3 SOLs	No culture (CSF)
9	SOL and meningitis	29	0	1.3	4.1	CT brain: 3 SOLs	Culture negative (CSF)
10	SOL and meningitis	2	0	2.3	2	CT brain: 3 SOLs	No culture (CSF)
11	SOL and meningitis	72	0	1.8	2	CT brain: 1 SOL	Culture negative (CSF)
12	SOL and meningitis	16	0	2.3	0.7	CT brain: 1 SOL	Culture negative (CSF)
13	Meningitis	45	0	1.1	1.5	CT brain: atrophy	Culture negative (CSF)
14	Meningitis	35	8	1.4	2.6	None	Culture negative (CSF)
15	Meningitis	28	74	0.7	1.6	CT brain: basal ganglia infarct	Culture negative (CSF)
16	Meningitis	253	31	5.1	2	CT brain: multiple SOLs	Culture negative (CSF)
17	Meningitis	13	6	0.7	2.3	CT brain: no SOL	Culture negative (CSF)
18	Meningitis	144	0	0.9	2.7	CT brain: no SOL	Culture negative (CSF)
19	Meningitis	5	2	1.6	0.4	CT brain: meningeal enhancement, 1 infarct	Culture negative (CSF)
20	Meningitis	30	0	2.3	2.2	CT brain: no SOL	<i>Mycobacterium tuberculosis</i> cultured (CSF)
21	Radiculomyelitis	23	2.9	None	Culture negative (CSF)
22	Radiculomyelitis	86	0	72	2.3	MRI spine: radiculomyelitis	Culture negative (CSF)
23	Spondylitis with radiculopathy	MRI spine: spondylitis	Culture negative (aspirate)

NOTE. ART, combination antiretroviral therapy; CSF, cerebrospinal fluid; CT, computed tomography; Glc, glucose; HR, isoniazid and rifampin; HRZE, isoniazid, rifampin, pyrazinamide, and ethambutol; HRZES, isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin; MRI, magnetic resonance imaging; NA, not ascertained; NP, not performed; SOL, space-occupying lesion; TMP-SMX, trimethoprim-sulfamethoxazole.

nadir was 50 cells/ μ L, and 19 weeks after starting antitubercular treatment, she initiated ART (stavudine 30 mg twice daily, lamivudine 150 mg twice daily, efavirenz 600 mg nightly). She received trimethoprim-sulfamethoxazole chemoprophylaxis (160 mg/800 mg twice daily). Sixteen days after initiation of ART, she developed headache, neck stiffness, and vomiting. Computed tomography of her brain showed a 10-mm partly solid, partly cystic, inhomogeneously enhancing lesion in the left temporo-parietal area (figure 2), with surrounding edema. Lumbar puncture showed an aseptic lymphocytic meningitis (table 2). Bacterial and fungal cultures had negative results, and the results of serological tests for syphilis performed on cerebrospinal fluid samples were negative. Although serological test results were positive for toxoplasmosis, the patient did not receive therapeutic trimethoprim-sulfamethoxazole (320 mg/1600 mg twice daily). We intensified antitubercular treatment from isoniazid and rifampin to isoniazid, rifampin, pyrazinamide, and ethambutol, and we prescribed prednisone (60 mg daily; 1.5 mg/kg/day) for 4 weeks, followed by a 4-week taper. The patient's headache and neck stiffness resolved. No subsequent deterioration occurred.

Patient 14: paradoxical TB-IRIS meningitis. A 33-year-old man with HIV infection received a diagnosis of miliary tuberculosis on the basis of chest radiograph findings. We were unable to obtain microbiological confirmation of tuberculosis, because sputum induction was unsuccessful. The patient had no history of tuberculosis and started treatment with isoniazid, rifampin, pyrazinamide, and ethambutol. He experienced improvement while receiving antitubercular treatment; cough, shortness of breath, and generalized weakness resolved. Sixteen days after starting antitubercular treatment, he initiated ART (stavudine, lamivudine, and efavirenz), because his CD4⁺ cell count nadir was 41 cells/ μ L. One day after initiation of ART, the patient developed headache, fever, and vomiting. On physical examination, he was febrile and tachycardic (heart rate, 128 beats per min) but had no nuchal rigidity. We performed a lumbar puncture, which revealed an aseptic lymphocytic meningitis (table 2). The patient continued to receive antitubercular treatment with isoniazid and rifampin and received prednisone (40 mg twice daily) for 16 days. His headache, fever, and vomiting resolved, and he experienced no subsequent deterioration.

Table 2. (Continued.)

TB regimen at neurologic deterioration	TB treatment intensified	Corticosteroids prescribed/duration, weeks	ART stopped	TMP-SMX chemoprophylaxis before deterioration (160/800 mg/day)	Toxoplasma serologic test results (IgG)	Therapeutic TMP-SMX (320/1600 mg twice daily) after deterioration	Syphilis serologic test result (site)	6-Month outcome ^b
HRZE	No	Yes/10 ^c	No	Yes	Positive	No	Positive (blood)	Alive ^d
HRZE	Yes	Yes/5	Yes	Yes	Negative	Yes	NP	Died
HR	Yes	Yes/49 ^c	Yes	Yes	Negative	Yes	Negative (blood)	Alive
HRE	No	Yes/13 ^c	No	Yes	Negative	Yes	Negative (blood)	Alive
HRZE	No	Yes/17 ^c	No	Yes	Positive	Yes	NP	Alive ^d
HR	No	No ^c	No	NA	Positive	Yes	Negative (blood)	Alive ^d
HR	Yes	Yes/4 ^c	No	Yes	Positive	Yes	Negative (blood)	Lost
HRZE	No	Yes/11 ^c	No	Yes	Positive	No	Negative (blood)	Alive ^d
HRZE	No	Yes/4 ^c	No	NA	Negative	No	Negative (CSF)	Alive
HR	Yes	Yes/1	Yes	Yes	Positive	Yes	Negative (CSF)	Died
HR	Yes	Yes/1 ^c	No	NA	NP	No	NP	Alive ^d
HR	Yes	Yes/8 ^c	No	Yes	Positive	No	Negative (CSF)	Alive
HRZES	No	Yes/1 ^c	No	Yes	NP	No	Negative (blood)	Alive
HRZE	No	Yes/2 ^c	No	Yes	NP	No	Negative (blood)	Alive ^d
HR	No	Yes/19 ^c	No	Yes	NP	No	Negative (blood)	Alive ^d
HRZE	No	Yes/10 ^c	No	Yes	NP	No	Negative (blood)	Alive
HRZE	No	Yes/1 ^c	No	Yes	NP	No	Negative (blood)	Alive ^d
HR	No	Yes/1 ^c	No	NA	NP	No	Negative (blood)	Lost ^d
HRZES	No	Yes/6 ^c	No	Yes	NP	No	Negative (CSF)	Died
HR	Yes	Yes/11 ^c	No	Yes	NP	No	Negative (CSF)	Alive ^d
HRZE	No	No	No	NA	NP	No	Negative (blood)	Lost
HRZE	No	Yes/8	No	NA	NP	No	NP	Lost
HR	No	Yes/22 ^c	No	NA	NP	No	NP	Alive

^a Normal CSF values were as follows: lymphocyte count, <5 cells/ μ L; polymorphonuclear cell count, <1 cell/ μ L; protein level, 0.15–0.45 g/L; and glucose level, 2.2–3.9 mmol/L.

^b Outcome 6 months after neurologic deterioration; Lost indicates lost to follow-up 6 months after neurologic deterioration.

^c Initial clinical improvement.

^d Full physical and mental recovery.

Patient 23: paradoxical TB-IRIS spondylitis complicated by radiculopathy.

A 31-year-old woman with HIV infection received a diagnosis of disseminated tuberculosis; she had constitutional symptoms, abdominal ultrasound visualized ascites and adenopathy, and we cultured *M. tuberculosis* that was susceptible to isoniazid and rifampin from her sputum samples. The patient had no history of tuberculosis and commenced treatment with isoniazid, rifampin, pyrazinamide, and ethambutol. She stopped coughing and gained weight, and her malaise and night sweats resolved. Her CD4⁺ cell count nadir was 99 cells/ μ L, and 24 weeks after starting antitubercular treatment, she initiated ART with stavudine, lamivudine, and efavirenz. Ten days after initiation of ART, the patient's symptoms of night sweats and anorexia recurred, and a chest radiograph revealed a new miliary infiltrate. Sixty-one days after ART initiation, the patient developed back pain radiating down her right leg. On physical examination, we found weakness of hip flexion and knee extension and loss of the right knee reflex but

no sensory deficit. The patient's clinical features were in keeping with a right L3/4 radiculopathy. Magnetic resonance imaging of her spine confirmed tuberculous spondylitis with an epidural component impinging the right aspect of the thecal sac (figure 2). We diagnosed a neurological manifestation of paradoxical TB-IRIS, and the patient initiated treatment with prednisone (60 mg daily; 1.5 mg/kg/day). Despite prednisone, her pain persisted. We performed a computed tomography-guided paraspinal fine-needle aspiration; on microscopic examination, the aspirate showed 1+ neutrophils, and on culture, it was negative for pyogenic bacteria, mycobacteria, and fungi. The patient received a total of 152 days of corticosteroid therapy, which was tapered on cessation. The patient's back pain improved with opiate and nonsteroidal anti-inflammatory drugs, but she had residual weakness and sensory loss affecting the lateral aspect of her right thigh. By 173 days after initiation of ART, the patient's CD4⁺ cell count had increased to 700 cells/ μ L, and her viral load was 500 copies/mL.

DISCUSSION

Our study is, to our knowledge, the first case series of neurologic TB-IRIS. We found that neurologic TB-IRIS accounts for >10% of paradoxical TB-IRIS cases in a hospital setting. We found a mortality rate of 13% (3 deaths among 23 patients) for neurologic TB-IRIS, but the mortality rate may have been 30% (7 deaths among 23 patients) if all 4 patients who were lost to follow-up died. Published data suggest that, in developing countries, many patients in antiretroviral programs who are lost to follow-up actually die [20]. In a Vietnamese report that described 44 patients with HIV-1 infection who received dexamethasone for tuberculous meningitis, 27 (61%) of 44 patients died [21]. Reasons for differences in mortality rates include nonavailability of ART in the Vietnamese study and the fact that the Vietnamese study assessed outcome at 9 months, not 6 months. In addition, in our study, by analyzing data for patients who were referred with TB-IRIS, we may have selected for a group of patients with neurologic tuberculosis who had a more favorable prognosis. Patients with more-severe and more-extensive neurologic tuberculosis may have died before receiving ART. Also, patients who developed neurologic TB-IRIS may have died before referral to our hospital. The published literature reports only 1 death due to neurologic TB-IRIS [9]; however, this is almost certainly because of a paucity of published case reports [9–13].

In our study, initial improvement of symptoms and/or signs occurred in 19 of 23 patients; 18 of these 19 patients with initial improvement received corticosteroids. Full physical and mental improvement occurred in 10 of 16 patients who were alive and in care at 6 months. A substantial number of patients (6 of 16 patients) had residual neurologic disability at 6 months that was likely to be permanent. A recent meta-analysis of published literature does not support or refute the use of corticosteroids to reduce death and neurologic deficit among patients with HIV-1 infection who have tuberculous meningitis [22]. Corticosteroids are proposed as a treatment for TB-IRIS, although available evidence is anecdotal [23, 24]. To date, no clinical trials show evidence to support the use of corticosteroids in treating TB-IRIS. We are unable to conclude from our observational study whether corticosteroids reduce mortality or disability in neurologic TB-IRIS.

To diagnose TB-IRIS, differential diagnoses need to be excluded or resolution should occur without treatment for other opportunistic infections [1]. This is difficult in cases involving space-occupying lesions because of limited access to brain biopsy. Differential diagnoses for TB-IRIS include cryptococcoma, progressive multifocal leukoencephalopathy IRIS [25], bacterial abscess, lymphoma, neurocysticerci, and syphilitic gumma. We were unable to definitively exclude all other differential diagnoses by means of brain biopsy; however, 10 of

12 patients with space-occupying lesions did not experience deterioration despite receiving no treatment for these other aforementioned illnesses.

The major differential diagnosis for tuberculoma IRIS, however, is cerebral toxoplasmosis. A subgroup of patients (7 of 12 patients) received simultaneous treatment for toxoplasmosis and tuberculosis in our study. In certain parts of South Africa, *Toxoplasma gondii* is isolated more commonly than *M. tuberculosis* from biopsy specimens and aspirates of intracranial mass lesions [26], whereas in other parts of South Africa, *M. tuberculosis* is isolated more commonly [27]. We anticipate a higher proportion of tuberculosis cases in South Africa, because the tuberculosis case notification rate in Cape Town is one of the highest in the world [15]. In addition, key features that suggest tuberculosis, rather than other infectious causes, include (1) concurrent presence of pulmonary tuberculosis or other non-neurologic tuberculosis and (2) basal meningeal enhancement [27]. All of the patients in our study whose tuberculosis diagnosis was microbiologically unconfirmed had radiologic features of non-neurologic tuberculosis. In addition, of 7 patients who were treated for possible cerebral toxoplasmosis, 3 had serologic test results that were negative for toxoplasmosis, and all 7 had radiologic evidence of non-neurologic tuberculosis. Although we had strong clinical suspicion of paradoxical neurologic TB-IRIS in these 7 patients, we opted to also treat for possible cerebral toxoplasmosis, because the clinical condition of these patients was severe, and a confirmatory diagnostic test for TB-IRIS is not yet available. Trimethoprim-sulfamethoxazole, used in our setting, and pyrimethamine-sulfadiazine, used elsewhere, have no significant difference with respect to clinical efficacy during short-term therapy for toxoplasmic encephalitis [28].

Patients without apparent neurologic tuberculosis before ART may present with neurologic TB-IRIS after ART initiation. This occurred in 17 of 23 patients in our case series. Presumably, this is attributable to subclinical seeding of *M. tuberculosis* into neurologic tissue that provokes an inflammatory response at the time of IRIS. Although mycobacterial culture results are usually negative at TB-IRIS diagnosis, *M. tuberculosis* may be cultured, particularly if IRIS occurs early during antitubercular treatment [1]. This occurred in 1 patient.

Incident MDR tuberculosis may occur in ~10% of HIV-1-infected patients with tuberculous meningitis [28]. Patients with MDR tuberculous meningitis have significantly poorer outcomes [29]. Previously, we diagnosed rifampin-resistant tuberculosis in >10% of patients with suspected TB-IRIS [2]. In this study, we diagnosed MDR tuberculosis in ~10% of patients with suspected neurologic TB-IRIS. We empirically intensified antitubercular treatment in 7 patients with paradoxical neurologic TB-IRIS while awaiting mycobacterial culture and drug

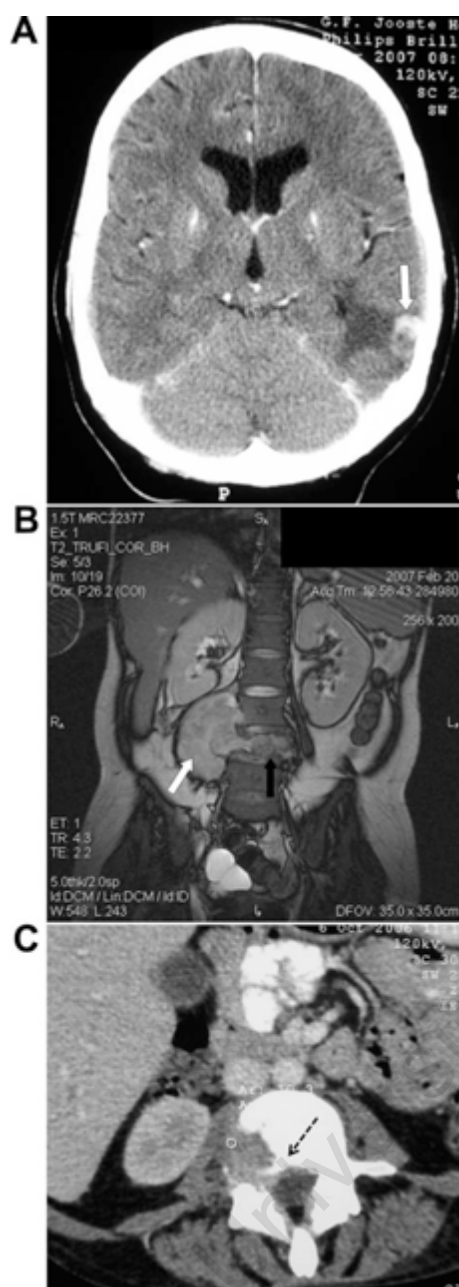


Figure 2. A, Paradoxical brain tuberculoma and meningitis immune reconstitution inflammatory syndrome (IRIS) in patient 12. Computed tomography of the patient's brain showed a 10-mm partly solid, partly cystic, inhomogeneously enhancing lesion in the left temporo-parietal region (white arrow). B, Paradoxical tuberculosis (TB)-associated IRIS spondylitis complicated by radiculopathy in patient 23. Magnetic resonance imaging of the patient's spine confirmed a right paravertebral collection (white arrow) with tuberculous spondylitis (black arrow). C, Computed tomography of patient 23 revealed the epidural component of the spondylitis lesion (dashed arrow) impinging the right aspect of the thecal sac.

susceptibility results; however, none of these patients cultured MDR tuberculosis. We are unable to ascertain the extent that intensified antitubercular treatment improved clinical outcome.

It is possible that the 7 patients who received a diagnosis of

HIV-1 encephalopathy and delirium (figure 1) may have experienced cognitive deterioration as a result of cerebral inflammation (cerebritis) due to TB-IRIS. However, in these patients, we did not diagnose neurologic TB-IRIS, because either (1) the patient did not have a space-occupying lesion and thus did not fulfill our case definition, or (2) the imaging modality that we used (computed tomography rather than magnetic resonance imaging in most cases) was not sensitive enough to detect all space-occupying lesions [30, 31]. This may be a limitation of our case definition and warrants further investigation.

We report a median duration of 14 days from ART initiation to the onset of TB-IRIS symptoms in both this study and our previous study of paradoxical TB-IRIS [2]. Other studies report a median duration from ART initiation to the onset of TB-IRIS symptoms of 12 days (IQR, 5–17 days) [32], 34 days (IQR, 8–97 days) [9], and 47 days (IQR, 36–81 days) [33]. Variability in baseline CD4⁺ cell counts and duration of antitubercular treatment before ART initiation may account for the discrepancy among studies, because these are risk factors for TB-IRIS [34, 35]. Median duration of hospitalization in patients with TB-IRIS is usually ~1 week [9], which is similar to our findings. In HIV-1-infected patients with tuberculous meningitis, median symptom duration before assessment varies from 11 to 18 days [36–38]. Our shorter median symptom duration of 6 days before assessment for neurologic TB-IRIS may indicate increased inflammation from IRIS. Differing referral patterns between our setting and other settings may also account for shorter symptom duration.

In a hospital setting, patients with suspected non-neurologic TB-IRIS are more likely than patients with suspected neurologic TB-IRIS to receive an eventual diagnosis of TB-IRIS. We attribute this finding to screening of patients by physicians at primary care clinics. It is likely that patients with suspected non-neurologic TB-IRIS with alternative illnesses to TB-IRIS were not assessed at our hospital, because they improved with appropriate treatment prescribed by primary care physicians. Intuitively, patients with suspected TB-IRIS with neurologic deterioration require rapid referral to the hospital for inpatient admission and investigation, thus increasing the likelihood of diagnosis of other illnesses.

Although paradoxical neurologic TB-IRIS is a potentially life-threatening manifestation of paradoxical TB-IRIS, there is most likely a spectrum of disease severity in neurologic TB-IRIS. Milder forms of neurologic TB-IRIS with spontaneous recovery and extremely severe neurologic TB-IRIS with rapid progression to death before referral may not have been included in our case series. We did not microbiologically confirm tuberculosis in 9 (39%) of 23 patients; however, tuberculosis is difficult to confirm microbiologically in neurological disease. *M. tuberculosis* was cultured from only 31% of patients with tuberculous meningitis in a Vietnamese study [21]. All 3 patients

who died with a diagnosis of paradoxical neurologic TB-IRIS did not have cultures positive for *M. tuberculosis*; thus, drug susceptibility testing to exclude MDR tuberculosis could not be performed. We obtained cerebrospinal fluid for analysis from 18 of 23 patients but could have improved diagnostic accuracy by performing autopsies and brain biopsies. Brain biopsies carry the risk of postoperative hemorrhage [39] and are difficult to access in our setting. Standardized tools were not used to assess neurocognitive function or neurologic outcomes. Rather, we relied on the bedside clinical assessment of the attending physician at hospital admission and discharge, as well as records from clinic follow-up. Lastly, this study was conducted at a referral hospital that served a large population with a high incidence of HIV-1-associated tuberculosis. Our findings may not be generalizable to primary care settings or settings where HIV-1-associated tuberculosis is less common.

In conclusion, in a hospital setting, neurologic TB-IRIS is a not-infrequent manifestation of TB-IRIS and causes considerable short-term morbidity but is associated with reasonable long-term outcomes. Future prospective studies may better (1) determine the incidence of neurologic TB-IRIS among patients with initial neurologic and non-neurologic tuberculosis disease; (2) determine optimal management strategies to reduce death, neurologic deficits, and disabilities; and (3) describe the pathophysiologic findings of neurologic TB-IRIS.

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RESEARCH ARTICLE

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Clinical deterioration during antituberculosis treatment in Africa: Incidence, causes and risk factors

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Abstract

Background: HIV-1 and *Mycobacterium tuberculosis* cause substantial morbidity and mortality. Despite the availability of antiretroviral and antituberculosis treatment in Africa, clinical deterioration during antituberculosis treatment remains a frequent reason for hospital admission. We therefore determined the incidence, causes and risk factors for clinical deterioration.

Methods: Prospective cohort study of 292 adults who initiated antituberculosis treatment during a 3-month period. We evaluated those with clinical deterioration over the following 24 weeks of treatment.

Results: Seventy-one percent (209/292) of patients were HIV-1 infected (median CD4+: 129 cells/ μ L [IQR:62-277]). At tuberculosis diagnosis, 23% (34/145) of HIV-1 infected patients qualifying for antiretroviral treatment (ART) were receiving ART; 6 months later, 75% (109/145) had received ART. Within 24 weeks of initiating antituberculosis treatment, 40% (117/292) of patients experienced clinical deterioration due to co-morbid illness ($n = 70$), tuberculosis related illness ($n = 47$), non AIDS-defining HIV-1 related infection ($n = 25$) and AIDS-defining illness ($n = 21$). Using HIV-1 uninfected patients as the referent group, HIV-1 infected patients had an increasing risk of clinical deterioration as CD4+ counts decreased [CD4+>350 cells/ μ L: RR = 1.4, 95% CI = 0.7-2.9; CD4+:200-350 cells/ μ L: RR = 2.0, 95% CI = 1.1-3.6; CD4+<200 cells/ μ L: RR = 3.0, 95% CI = 1.9-4.7]. During follow-up, 26% (30/117) of patients with clinical deterioration required hospital admission and 15% (17/117) died. Fifteen deaths were in HIV-1 infected patients with a CD4+<200 cells/ μ L.

Conclusions: In multivariate analysis, HIV-1 infection and a low CD4+ count at tuberculosis diagnosis were significant risk factors for clinical deterioration and death. The initiation of ART at a CD4+ count of <350 cells/ μ L will likely reduce the high burden of clinical deterioration.

Background

Adherence to antituberculosis treatment in advanced human immunodeficiency virus type 1 (HIV-1) infection results in rapid sterilisation of sputum, radiographic improvement and a low risk of relapse [1]. The benefits of antiretroviral treatment (ART) in reducing HIV-1 replication and restoring pathogen-specific immunity are well described [2,3]. Despite the availability of antituberculosis and antiretroviral treatment in Africa, clinical deterioration during antituberculosis treatment in HIV-1

infected patients remains an important reason for hospital admission and death [4,5].

Profoundly immune-suppressed HIV-1 infected patients may encounter a complicated clinical course after starting antituberculosis treatment. While initiation of ART during antituberculosis treatment reduces mortality [6], the optimal interval from antituberculosis treatment to initiation of ART is not known. Early initiation of ART restores pathogen-specific immunity, but also significantly increases the risk of the tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) [7]. Conversely, a delay in initiation of

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ART may allow additional AIDS-defining illnesses to manifest. Other reasons for clinical deterioration during antituberculosis treatment include antimicrobial resistance, suboptimal antituberculosis drug concentrations, drug reactions, and other opportunistic illnesses [8,9].

The greatest burden of HIV-1 infection and tuberculosis occurs in resource-limited settings, such as South Africa, where health systems are overwhelmed and rapid diagnostic tools are not readily available. In 2006, 337,400 of 482,000 tuberculosis patients in South Africa were HIV-1 co-infected and 105,000 tuberculosis deaths were reported [10]. The fatal consequences of HIV-1/*Mycobacterium tuberculosis* co-infection are well described [11,12]. However, the incidence of clinical deterioration during antituberculosis treatment amongst HIV-1 infected patients (compared to HIV-1 uninfected patients) is unknown. Determining the causes and risk factors for clinical deterioration during antituberculosis treatment may inform initiatives to reduce the burden on both tuberculosis and ART programmes.

In this study, we assessed patients at initiation of antituberculosis treatment and followed them for 24 weeks, in order to determine the incidence, causes and risk factors for clinical deterioration. We also discuss initiatives to reduce the high burden of clinical deterioration in resource-limited settings.

Methods

We conducted a prospective cohort study at Khayelitsha Site B tuberculosis clinic (Cape Town, South Africa) from 1 June 2008 through 15 February 2009. We assessed adult (≥ 18 years age) patients diagnosed with tuberculosis at Khayelitsha Site B tuberculosis clinic from 1 June 2008 through 31 August 2008 (3-month assessment period). Informed consent was obtained from all enrolled patients. HIV-1 voluntary counselling and testing is offered to all patients diagnosed with tuberculosis at Site B Khayelitsha. Patients were followed for 24 weeks from initiation of antituberculosis treatment. Our study was nested within a tuberculosis drug susceptibility testing (DST) survey in which first-line DST (for isoniazid and rifampin) was routinely performed, regardless of HIV-1 status or previous tuberculosis. The Research Ethics Committee of the University of Cape Town approved this study (REC 178/2008).

Study site and setting

Khayelitsha Site B tuberculosis clinic is a primary-level outpatient health care facility, which serves ~100,000 people within its high-density, low-income catchment area. Approximately 1,200 adult cases of tuberculosis are diagnosed per annum at Site B tuberculosis clinic. In 2006, the antenatal HIV-1 seroprevalence in this community was 33% (95%: 29.1 - 36.9%) [13]. According

to national protocol, patients with a new diagnosis of tuberculosis receive 6 months of daily antituberculosis treatment (isoniazid, rifampin, pyrazinamide, and ethambutol for 2 months, followed by isoniazid and rifampin for 4 months) [14]. The retreatment regimen is: isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin for 2 months, followed by isoniazid, rifampin, pyrazinamide, and ethambutol for 1 month, and then isoniazid, rifampin, and ethambutol for 5 months. First-line ART in South Africa is stavudine, lamivudine, and either nevirapine or efavirenz. Efavirenz is preferred for patients who are receiving rifampin-based antituberculosis treatment. Patients with a CD4+ cell count <200 cells/ μ L and/or a history of a World Health Organization (WHO) stage 4 illness, other than extra-pulmonary tuberculosis, are eligible to commence ART [15]; ART is typically commenced 2 months after initiation of antituberculosis treatment. If the CD4+ cell count is <50 cells/ μ L or a serious HIV-1-related illness is present, ART may be commenced 2 weeks after starting antituberculosis treatment [15]. To date, >5,000 people have initiated ART at Khayelitsha Site B HIV clinic.

Definitions

"Tuberculosis patients" refers to patients diagnosed with tuberculosis and initiated on antituberculosis treatment. We defined microbiologically-confirmed tuberculosis as *Mycobacterium tuberculosis* cultured or acid-fast bacilli visualised in a biological specimen. Biological specimens included sputum, pleural fluid, urine, nodal aspirates, pericardial aspirates and cerebrospinal fluid. We defined microbiologically-unconfirmed tuberculosis according to the WHO case definitions for smear-negative and extra-pulmonary tuberculosis [16]. Biological specimens were obtained at tuberculosis diagnosis, at 8 and 20 weeks' follow-up and at clinical deterioration. First-line DST using the GenoType MTBDRplus assay [17] was performed on biological specimens that were smear-positive for acid-fast bacilli and/or culture positive for *Mycobacterium tuberculosis*. We defined multidrug-resistant (MDR) tuberculosis as *M. tuberculosis* resistant to isoniazid and rifampin.

We defined clinical deterioration as symptomatic worsening or failure to stabilise within 24 weeks following initiation of antituberculosis treatment. We subdivided the causes of clinical deterioration into HIV-1 related illnesses and HIV-1 unrelated illnesses. HIV-1 related causes included AIDS defining illnesses (according to WHO stage 4 criteria [18]), and non AIDS-defining illnesses i.e. Non-AIDS defining HIV-1 related infections. HIV-1 unrelated illnesses included tuberculosis related illnesses, and illnesses unrelated to tuberculosis i.e. comorbid illnesses. Tuberculosis-related illnesses included MDR-TB, deterioration due to poor adherence,

paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) and paradoxical reactions. A co-morbid illness was an acute illness that occurred after tuberculosis diagnosis and was considered not to be directly attributable to HIV-1 or tuberculosis. A co-morbid illness also included an acute deterioration of a pre-existing condition. For example, we regarded diabetic ketoacidosis in a diabetic patient as a co-morbid illness.

We defined paradoxical TB-IRIS according to the consensus clinical case definition for resource-limited settings [7]. We defined paradoxical reactions using an adaptation of this consensus clinical case definition of paradoxical TB-IRIS [7]. Three criteria were required for a diagnosis of paradoxical reaction:

1. Diagnosis of tuberculosis (microbiologic confirmation or according to WHO criteria [16]) with initial response to antituberculosis treatment,
2. The recurrence/new onset of tuberculosis disease manifestations within 24 weeks of antituberculosis treatment, and
3. Exclusion of alternative explanations for clinical deterioration (such as antituberculosis drug resistance, poor adherence, drug toxicity or reaction, or an additional infection).

Paradoxical reactions were diagnosed in both HIV-1 infected and uninfected patients. HIV-1 infected patients receiving ART at clinical deterioration were diagnosed with paradoxical TB-IRIS rather than a paradoxical reaction according to our definitions.

We defined a patient as being lost to follow-up if we were unable to trace a patient 24 weeks after initiation of antituberculosis treatment. We used clinic and hospital medical notes, as well as the National Health Laboratories Service database to trace specimens and the Provincial Government of the Western Cape's electronic tuberculosis register to trace patients.

Assessment of patients with clinical deterioration

The study was discussed with physicians and nurses at the tuberculosis clinic prior to commencement of the study. Adult patients with clinical deterioration within 24 weeks of initiation of antituberculosis treatment were prospectively evaluated (regardless of severity of illness, eventual diagnosis, or inpatient or outpatient management) to determine the reason for clinical deterioration. Stable patients were assessed at Khayelitsha Site B tuberculosis clinic; patients requiring hospital admission or invasive outpatient procedures were assessed at GF Jooste Hospital (the clinic's referral hospital). At clinical deterioration, we routinely obtained biological specimens to investigate drug-resistant *M. tuberculosis*. Specimens were also cultured for bacterial organisms.

Data collection and analysis

Data collected included demographic information, tuberculosis specimen results, HIV-1 status, antiretroviral treatment, CD4+ cell count at tuberculosis diagnosis, diagnosis at clinical deterioration and outcome 24 weeks after initiation of antituberculosis treatment. Statistical analyses were performed using Stata 10.0 (Texas, USA). Wilcoxon rank-sum and Kruskal-Wallis tests were used for group comparisons, and Fisher's exact tests for proportion comparisons. Variables with the outcome of interest were entered into Cox proportional hazards models to assess the independent effects of covariates. We censored patients at clinical deterioration when performing Cox-proportional hazards model analysis and relative risk calculations. Significant variables were removed from the model to assess whether these effects remained. The assumptions of the Cox model were verified; non-informative censoring was performed and the tests for the proportional hazards assumption were not significant. We censored patients at loss to follow-up when determining the incidence rate of clinical deterioration (illnesses diagnosed per 100 months of follow-up). Individual patients could contribute more than one event in the calculation of the incidence rate.

Results

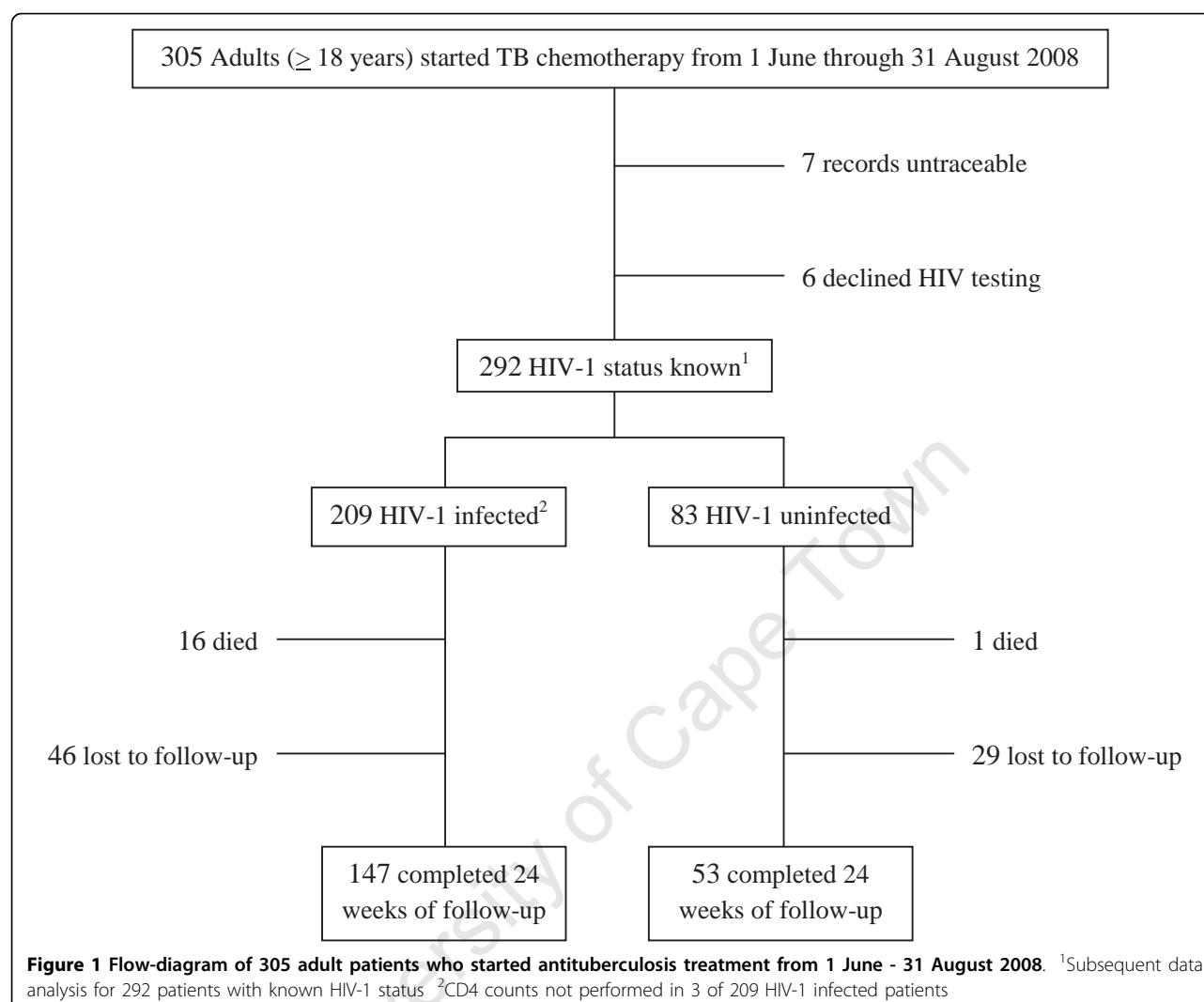
During the 3-month assessment period (figure 1), 305 adults (≥ 18 years age) initiated antituberculosis treatment, 7 of whom had untraceable clinical records and 6 of whom declined HIV-1 testing. We restricted our data analysis to the 292 (96%) patients whose HIV-1 status and clinic records were available. In 209 HIV-1 infected and 83 HIV-1 uninfected patients, loss to follow-up (46 [22%] of 209 vs. 29 [35%] of 83, p -value = 0.026) and mortality (16 [8%] of 209 vs. 1 [1%] of 83, p -value = 0.048) differed significantly at 24 weeks.

At initial tuberculosis diagnosis (table 1), HIV-1 infected patients were more likely than HIV-1 uninfected patients to be female, be of younger age, have extra-pulmonary tuberculosis and be diagnosed with tuberculosis at the referral hospital. HIV-1 uninfected patients were more likely than HIV-1 infected patients to have microbiologic confirmation of tuberculosis at initial tuberculosis diagnosis and during the 24 weeks of follow-up.

Prior to tuberculosis diagnosis, 34 (23%) of 145 HIV-1 infected patients who qualified for ART under national guidelines were receiving ART. Six months later, 109 (75%) of 145 patients had received ART.

Causes of Clinical Deterioration and Hospital Admission

During the 24 weeks of follow-up, 117 (40%, 95% CI: 35-46%) of 292 tuberculosis patients experienced clinical deterioration, of whom 101 were HIV-1 infected and 16



were HIV-1 uninfected. Causes of clinical deterioration (table 2) included: co-morbid illnesses (70 patients), tuberculosis-related illnesses (47 patients), non AIDS-defining HIV-1 related infections (25 patients) and AIDS-defining illnesses (21 patients). Peripheral neuropathy, enteric illness and deep venous thrombosis were frequent co-morbid illnesses. TB-IRIS and paradoxical reactions were frequent tuberculosis-related illnesses. Oesophageal candida, *Pneumocystis jirovecii* pneumonia and cryptococcal meningitis were frequent AIDS-defining illnesses. Of 117 patients who experienced deterioration, 30 (26%) required hospital admission [27 (27%) of 101 HIV infected and 3 (19%) of 16 HIV-1 uninfected patients (p-value = 0.756)]. Causes of inpatient hospital admission were paradoxical reaction or TB-IRIS (9 patients), new AIDS-defining illness (8 patients), deep venous thrombosis (6 patients), MDR-TB (2 patients), cardiomyopathy (1 patient), pneumothorax (1 patient), symptomatic deterioration

due to poor adherence with antituberculosis treatment (1 patient), hyperglycaemic emergency (1 patient) and seizure disorder (1 patient).

Risk Factors for Clinical Deterioration

In the 292 tuberculosis patients, 4 factors were significantly associated with clinical deterioration in univariate analysis: HIV-1 infection, diagnosis of tuberculosis at the referral hospital, evidence of extra-pulmonary tuberculosis, and absence of a DST result at tuberculosis diagnosis. Only HIV-1 infection (figure 2a) remained significant in multivariate analysis (adjusted hazard ratio [aHR] = 2.0, 95% CI = 1.1-3.6).

In subsequent analysis (figures not shown), we assessed whether the probability of clinical deterioration from non-HIV-1 related causes was associated with HIV-1 infection. In univariate analysis, we found a significant association between HIV-1 infection and non-HIV-1 related causes for deterioration (RR = 1.3, 95%

Table 1 Baseline characteristics and microbiologic confirmation of tuberculosis in 209 HIV-1 infected and 83 HIV-1 uninfected patients receiving antituberculosis treatment

	HIV-1 infected (n = 209)	HIV-1 uninfected (n = 83)	p-value
Female, n (%)	112 (54)	25 (30)	<0.001
Age in years, median (IQR)	35 (30-41)	38 (29-49)	0.035
CD4 count at TB diagnosis, n (%):			
>350 cells/mm ³	36 (17)	-	
200 - 350 cells/mm ³	37 (18)	-	
< 200 cells/mm ³	133 (64)	-	
Not performed	3 (1)	-	
Previous TB, n (%)	71 (34)	19 (23)	0.070
TB diagnosed at referral hospital, n (%):	79 (38)	13 (16)	<0.001
Microbiologic confirmation at TB diagnosis ¹ , n (%)	123 (59)	68 (82)	<0.001
Drug susceptibilities known at TB diagnosis ¹ , n (%)	67 (32)	49 (59)	<0.001
Culture sent at TB diagnosis, n (%)	169 (81)	68 (82)	1.000
Extra-pulmonary TB, n (%):	82 (39)	14 (17)	<0.001
Weight at TB diagnosis in kg, median (IQR)	54 (49-62)	54 (49-64)	0.644
Microbiologic confirmation at TB diagnosis and during 24 weeks of follow-up ² , n (%)	153 (73)	70 (84)	0.048
Drug susceptibilities known at TB diagnosis and during 24 weeks of follow-up ² , n (%)	117 (56)	57 (69)	0.296
Culture sent at TB diagnosis and/or during 24 weeks of follow-up, n (%)	190 (91)	76 (92)	1.000

Key:

¹within 14 days of TB diagnosis. No TB specimens sent in 12 HIV-1 infected patients and 3 HIV-1 uninfected patients at TB diagnosis.

²no TB specimens sent in 2 HIV-1 infected patients.

TB = tuberculosis, IQR = inter-quartile range, OR = odds-ratio, 95% CI = 95% confidence interval.

CI: 1.07 - 1.50). However, in the Cox proportional hazards model, using the same variables as figure 2a, this significant association was not confirmed (HR = 1.5, 95% CI: 0.81-2.64). This analysis suggests that HIV-1 infection is a significant variable for clinical deterioration because of HIV-1 related illnesses (either AIDS- or non AIDS-defining illnesses).

In the 209 HIV-1 infected tuberculosis patients, 3 factors were significantly associated with clinical deterioration in univariate analysis: a lower CD4+ count, diagnosis of tuberculosis at the referral hospital, and antiretroviral treatment received during antituberculosis treatment. Only a lower CD4+ stratum at tuberculosis diagnosis (figure 2b) remained significant in multivariate analysis (aHR = 1.5, 95% CI = 1.1-2.2).

In subsequent analysis (figures not shown), we assessed whether the probability of clinical deterioration from non-HIV-1 related causes was associated with decreasing CD4+ counts. We did not find a significant association between decreasing CD4+ count strata and non-HIV-1 related causes in both the univariate analysis (P = 0.189) and the Cox proportional hazards model (HR = 1.3, 95%CI: 0.88 - 1.97). This analysis suggests that decreasing CD4+ counts are a significant risk factor for clinical deterioration because of their association with HIV-1 related illnesses (either AIDS- or non AIDS-defining illnesses).

Relative Risk and Incidence Rate of Clinical Deterioration

HIV-1 infection and a low CD4+ count were the only significant risk factors for clinical deterioration in multivariate analysis. We therefore determined the relative risk and incidence rate of clinical deterioration according to HIV-1 status and CD4+ stratum. HIV-1 infected patients were more likely than HIV-1 uninfected patients to experience clinical deterioration (RR = 2.6, 95%CI: 1.6-4.0). Using HIV-1 uninfected patients as the referent group, the relative risk (RR) of clinical deterioration increased as the CD4+ counts in HIV-1 infected patients decreased (CD4+ >350 cells/μL: RR = 1.4, 95% CI = 0.7-2.9; CD4+200-350 cells/μL: RR = 2.0, 95% CI = 1.1-3.6; CD4+ <200 cells/μL: RR = 3.0, 95% CI = 1.9-4.7). The incidence rate (IR) of clinical deterioration (illnesses diagnosed per 100 months of follow-up) also increased as the CD4+ counts decreased. Incidence rates differed significantly between HIV-1 uninfected patients (IR = 5.1, 95% CI = 3.1-7.5) and HIV-1 infected patients with a CD4+ count of 200-350 cells/μL (IR = 12.3, 95% CI = 8.2-17.0). Similarly, incidence rates differed significantly between HIV-1 infected patients with a CD4+ count of 200-350 cells/μL and HIV-1 infected patients with a CD4+ count < 200 cells/μL (IR = 20.7, 95% CI 17.8-23.9).

Figure 3 is a Lowess plot showing the proportion of patients who experienced clinical deterioration during

Table 2 Illnesses (n = 199) in 101 HIV-1 infected and 16 HIV-1 uninfected patients who experienced clinical deterioration during 24 weeks of antituberculosis treatment

	HIV-1 infected	HIV-1 uninfected
number of illnesses = n, (total number of patients = pnts)	180 (101)	19 (16)
1. Tuberculosis related illnesses, n (pnts)	49 (43)	5 (5)
TB-IRIS	22	-
Paradoxical reaction	17	4
MDR-TB ¹	4	1
Deterioration due to poor adherence	5	-
Low rifampin concentration in comparison to the recommended range	1	-
2. AIDS-defining illnesses, n (pnts)	29 (21)	-
Oesophageal candida	11	-
Pneumocystis jirovecii pneumonia ²	5	-
Cryptococcal meningitis	4	-
Cytomegalovirus retinitis	3	-
Other ³	6	-
3. Non-AIDS defining HIV-1 related infections, n (pnts)	27 (25)	-
Superficial herpes infection ⁴	9	-
Bacterial infection ⁵	9	-
Fungal (oral/vaginal/superficial) infection	9	-
4. Co-morbid illnesses, n (pnts)	76 (59) ⁶	14 (11) ⁷

Key:

TB = tuberculosis; TB-IRIS = TB-associated immune reconstitution inflammatory syndrome

¹MDR-TB: *Mycobacterium tuberculosis* resistant to rifampin and isoniazid

²All 5 patients diagnosed with Pneumocystis jirovecii pneumonia received trimethoprim sulfamethoxazole chemoprophylaxis (160/800 mg daily).

³Other = HIV-1 associated nephropathy (2), HIV-1 associated encephalopathy (2), Disseminated Kaposi's sarcoma (2)

⁴5 patients diagnosed with herpes simplex, 4 patients diagnosed with herpes zoster (all < 1 month duration)

⁵Urinary tract infection (3) - *E. coli*, *Klebsiella sp.*, enterococcus; Pelvic inflammatory disease (3); *H. influenza* pneumonia (1); otitis media (1); carbuncle (1)

⁶76 illnesses diagnosed in 59 patients:

CD4 > 350 cells/mm³ = 7 illnesses diagnosed in 7 patients: deep venous thrombosis (2); sacroiliitis (1); acute asthmatic attack (1); cardiomyopathy (1); severe epistaxis (1); supra-condylar fracture (1),

CD4 200 - 350 cells/mm³ = 12 illnesses diagnosed in 7 patients: enteric illness with no pathogen isolated (4); hyperglycaemic emergency (2); seizures, cause not determined (2); acute asthmatic attack (1); efavirenz side-effect (1); deep venous thrombosis (1); haemorrhoids/fistula-in-ano (1),

CD4 < 200 cells/mm³ = 56 illnesses in 44 patients: peripheral neuropathy (12); enteric illness with no pathogen isolated (8); deep venous thrombosis (7); dermatitis (4); cerebrovascular accident (3); scabies (3); minor traumatic injury (3); pneumothorax (2); pancreatitis (1); cardiomyopathy (1); miscarriage (1); cryptococcal IRIS (immune reconstitution inflammatory syndrome) adenitis (1); drug induced hepatitis (1); cause not determined but patient subsequently improved (9).

⁷14 illnesses diagnosed in 11 patients: Drug side effect (3) - hydrochlorothiazide (1), pyrazinamide (1), kanamycin (1); hyperglycaemic emergency (2); post-TB bronchiectasis (1); peripheral neuropathy (1); pulmonary silicosis (1); miscarriage (1); cause not determined but patient subsequently improved (5)

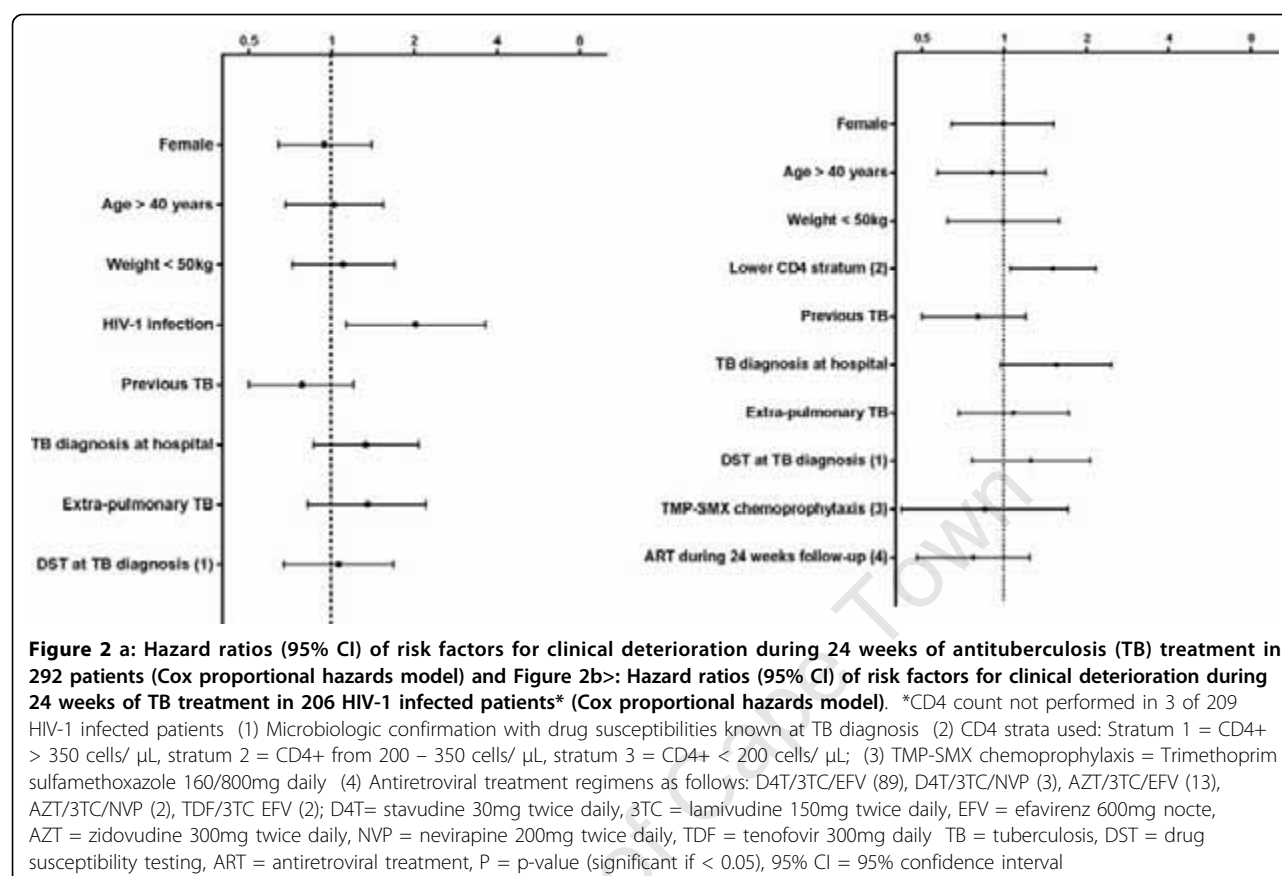
the 24 weeks of antituberculosis treatment. The initial peak at 6 weeks in HIV-1 uninfected patients corresponds with tuberculosis-related illnesses (mostly paradoxical reactions) and the baseline fluctuations represent co-morbid illnesses. The curve for HIV-1 infected patients with a CD4+ count > 350 cells/μL is similar to that of HIV-1 uninfected patients, despite an earlier peak for paradoxical reactions. The proportion of HIV-1 infected patients with a CD4+ count of 200-350 cells/μL who experienced clinical deterioration substantially increased after 10 weeks of follow-up. Furthermore, a substantially higher proportion of HIV-1 infected patients with a CD4+ count <200 cells/μL experienced clinical deterioration compared to other CD4+ strata.

Mortality

Fifteen of 17 deaths occurred in HIV-1 infected patients with a CD4+ count < 200 cells/μL. The median interval from antituberculosis treatment to death was 98 days (IQR = 59 -128). Eight of 17 deaths occurred between 10 and 20 weeks of follow-up. Diagnoses at time of death included: AIDS-defining illnesses (5), poor adherence with antituberculosis treatment (4), paradoxical neurologic TB-IRIS (3), enteric illness (2), MDR-TB (1), pulmonary embolus (1) and tension pneumothorax (1).

Loss to follow-up

Within 24 weeks of commencing antituberculosis treatment, 75 patients were lost to follow-up. The median interval from antituberculosis treatment to loss to



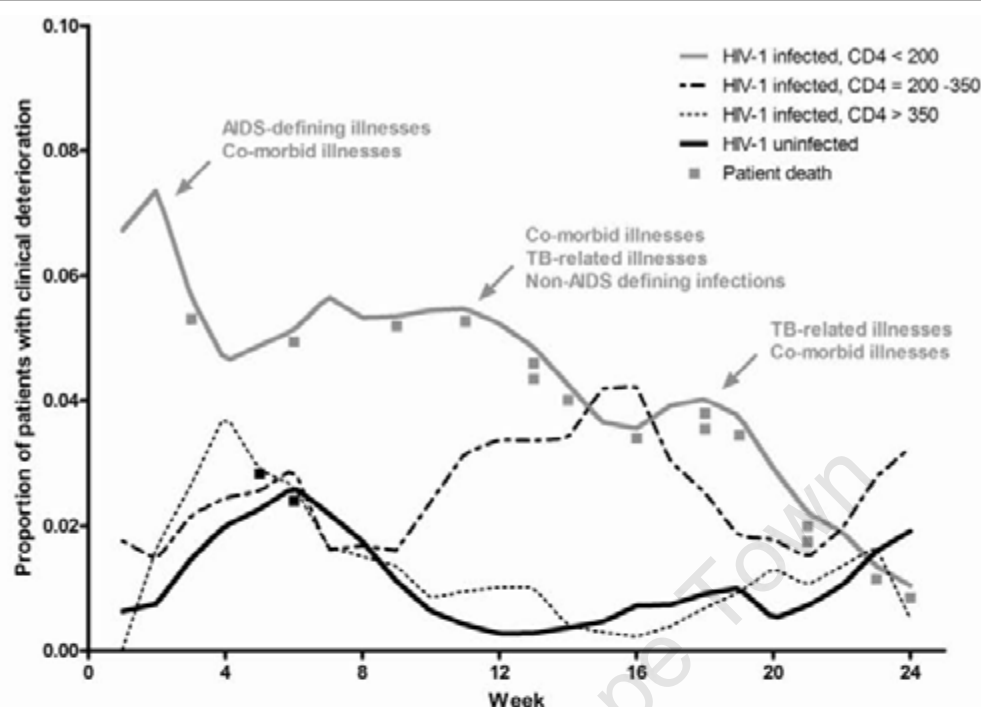
follow-up was 82 days (IQR = 48 -125). The CD4+ counts of 46 HIV-1 infected patients who were lost to follow-up (median 150 cells/ μ L, IQR = 67-347) did not differ significantly ($P = 0.362$) from the CD4+ counts of 147 HIV-1 infected patients who were not lost to follow-up was (median 144 cells/ μ L, IQR = 66-272).

Discussion

Khayelitsha Site B TB clinic illustrates the successful integration of public HIV-TB health-care services in South Africa. These services include voluntary counseling and testing of HIV status in >95% of TB patients, drug susceptibility testing on almost all bacteriologic specimens, trimethoprim-sulfamethoxazole chemoprophylaxis in >85% of HIV-1 infected patients, and aggressive ART initiation in HIV-1 infected patients according to national guidelines. Despite these measures, clinical deterioration remains an important clinical entity. During the 24 weeks of follow up, 40% of patients experienced clinical deterioration. In multivariate analyses, significant risk factors for clinical deterioration were HIV-1 infection and a low CD4+ count at tuberculosis diagnosis. Currently, HIV-infected patients present to health care services when they are already profoundly immune-suppressed. This results in a high burden of

tuberculosis, which is accompanied by multiple complications during antituberculosis treatment.

A distinct pattern of clinical deterioration emerged during the 24 weeks of follow-up (Figure 3). After 10 weeks of follow-up, we observed a rise in the proportion of patients with a CD4+ count of 200-350 cells/ μ L who experienced clinical deterioration. Few of these patients had initiated ART (according to national protocol [15]). Further studies are needed to determine whether ART initiated soon after tuberculosis diagnosis in this subgroup could reduce the incidence of clinical deterioration. A triple wave of illnesses occurred in profoundly immune-suppressed HIV-1 infected patients (CD4+ count < 200 cells/ μ L). The first wave (0-4 weeks) comprised co-morbid illnesses and AIDS-defining illnesses (data not presented). This highlights the profound immune-suppression at tuberculosis diagnosis and the rapid occurrence of AIDS-defining illnesses soon thereafter. The second wave represented co-morbid illnesses, tuberculosis-related illnesses and non AIDS-defining HIV-1 related infections, while the third wave included tuberculosis-related and co-morbid illnesses. This demonstrates that immune restoration during antituberculosis treatment and ART is not without complications [7]. The substantial occurrence of co-morbid illnesses throughout the 24 weeks of follow-up was



Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Number in follow-up																								
HIV-1 uninfected	79	76	76	74	74	72	72	71	69	68	66	65	64	63	62	60	59	59	57	57	56	55	54	53
HIV-1 infected, CD4+ > 350	36	35	35	35	33	33	33	31	31	31	31	30	30	30	29	28	28	28	28	26	24	24	24	24
HIV-1 infected, CD4+ = 200-350	37	37	37	37	37	37	37	36	36	36	36	36	36	36	35	34	32	32	32	32	32	32	32	31
HIV-1 infected, CD4+ < 200	132	129	126	126	124	125	125	125	122	116	116	114	112	111	109	107	106	103	100	99	96	94	93	92
Number with deterioration																								
HIV-1 uninfected	1	0	1	2	1	3	2	2	1	0	0	0	0	0	0	1	0	0	2	0	0	0	1	1
HIV-1 infected, CD4+ > 350	0	1	0	3	1	2	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0
HIV-1 infected, CD4+ = 200-350	1	0	1	1	0	3	0	1	0	0	1	2	2	0	1	3	1	1	0	1	0	0	1	1
HIV-1 infected, CD4+ < 200	6	12	10	3	4	3	10	7	6	6	7	6	6	6	4	2	3	4	6	4	1	2	1	1
Percentage with deterioration																								
HIV-1 uninfected	1.3	0.0	1.3	2.7	1.4	4.2	2.8	2.8	1.4	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	3.5	0.0	0.0	0.0	1.9	1.9
HIV-1 infected, CD4+ > 350	0.0	2.9	0.0	8.6	3.0	6.1	0.0	0.0	3.2	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	3.8	0.0	0.0	0.0	4.2	0.0
HIV-1 infected, CD4+ = 200-350	2.7	0.0	2.7	2.7	0.0	8.1	0.0	2.8	0.0	0.0	2.8	5.6	5.6	0.0	2.9	8.8	3.1	3.1	0.0	3.1	0.0	0.0	3.1	3.2
HIV-1 infected, CD4+ < 200	4.5	9.3	7.9	2.4	3.2	2.4	8.0	5.6	4.9	5.2	6.0	5.3	5.4	5.4	3.7	1.9	2.8	3.9	6.0	4.0	1.0	2.1	1.1	1.1
Number of deaths																								
HIV-1 uninfected	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV-1 infected, CD4+ > 350	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV-1 infected, CD4+ = 200-350	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV-1 infected, CD4+ < 200	0	0	1	0	0	1	0	0	1	0	1	0	2	1	0	1	0	2	1	0	2	0	1	1

Figure 3 Lowess plot showing the proportion of patients who experienced clinical deterioration during 24 weeks of antituberculosis treatment.

unexpected. Hepatic, renal and cardiovascular related morbidity and mortality are well described in HIV-infected patients [19]. Similarly, co-morbid illnesses causing death in HIV-1 uninfected tuberculosis patients have also been reported [20]. However, the magnitude of co-morbid illnesses in HIV-1 infected patients receiving

antituberculosis treatment has not previously been reported. The plethora of illnesses and the 15 deaths (related to AIDS-defining illnesses, poor adherence, and neurologic TB-IRIS) suggest that the management of tuberculosis in profoundly immune-suppressed HIV-1 infected patients is complicated.

Preventing the transmission of HIV-1 and preserving pathogen-specific immunity in those already infected with HIV-1 (by initiating ART at higher CD4+ counts) will likely reduce the incidence of tuberculosis. The potential benefits include fewer deaths and less difficulty in managing complex drug interactions related to rifampin. However, the massive scale-up of ART to treat patients at higher CD4+ counts would require considerable financial and medical resources. In South Africa, less than 50% of patients who qualify for ART under current South African guidelines receive ART [15]. Due to the observational nature of our study, we were unable to compare the incidence of clinical deterioration after ART initiation in two groups of tuberculosis patients: those with a CD4+ count of 200-350 cells/ μ L and those with a CD4+ count < 200 cells/ μ L. Only patients in the latter group were eligible for ART according to South African guidelines [15].

Fewer deaths than anticipated occurred during the first 8 weeks [5]; many deaths (8 of 17) occurred from weeks 10 to 20 of follow-up. It is possible that patients with severe tuberculosis may have died in the referral hospital, precluding enrolment in our study. Patients requiring hospital admission for fatal AIDS-defining illnesses at tuberculosis diagnosis would similarly have not been enrolled. Ninety-two (32%) of 292 patients were diagnosed with tuberculosis at hospital, suggesting that tuberculosis disease at tuberculosis diagnosis was severe.

Bacterial pneumonia is an important cause of hospitalisation and death in HIV-1 infected patients with [21], or without [22] a diagnosis of tuberculosis. In our clinic-based cohort, only one patient with pneumonia cultured a bacterial organism (*Haemophilus influenzae*). We attribute this finding to the combined antibacterial properties of rifampin and trimethoprim-sulfamethoxazole (TMP-SMX) [23]. Most HIV-1 infected patients (86%) in our study received TMP-SMX chemoprophylaxis (160/800 mg daily).

We diagnosed symptomatic drug-induced hepatitis in one patient. Drug-induced hepatitis, defined as a 5-fold rise in liver enzymes (AST or ALT), is reported in 2-28% of patients receiving antituberculosis treatment [24] and 6% of HIV-1 infected patients receiving both antituberculosis and antiretroviral treatment [25]. In our study, liver function tests were not performed in asymptomatic patients receiving antituberculosis treatment or efavirenz-based ART, according to routine practice. Liver function tests were performed when patients experienced clinical deterioration.

During the course of our study, DST was performed at tuberculosis diagnosis, at 8 and 20 weeks of follow-up and at clinical deterioration. This differs from routine practice in South Africa where only certain patients (such as those receiving retreatment for tuberculosis

and those who do not respond to antituberculosis treatment) [14] receive DST due to resource constraints. The ability to perform DST in all patients in our study, regardless of previous tuberculosis, likely expedited diagnosis and appropriate management of MDR-TB, especially where the differential diagnoses included MDR-TB and TB-IRIS.

Our study has some limitations. Our definition of clinical deterioration (symptomatic worsening or failure to stabilise within 24 weeks after initiation of antituberculosis treatment) did not include episodes of clinical deterioration that occurred after 24 weeks of follow-up. Twenty-four weeks of follow-up is a short period of observation. It is possible that the causes for deterioration could differ after 24 weeks of follow-up. The relatively low number of patients diagnosed with MDR-TB during follow-up ($n = 5$) may underestimate the incidence of MDR-TB presenting after 24 weeks. It is noteworthy that 6 patients were diagnosed with MDR-TB during the 24 weeks of follow-up without fulfilling our definition of clinical deterioration. We have previously described the phenomenon of initial clinical improvement with rifampin-resistant *M. tuberculosis* despite receiving standard antituberculosis treatment [26]. At diagnosis of MDR-TB in these 6 patients, we appropriately intensified their antituberculosis treatment and no subsequent clinical deterioration occurred. We also excluded isolated radiological worsening from our case definition of clinical deterioration. Thus, our finding of clinical deterioration in 40% of patients may be an underestimate. Also, in 14 episodes the cause of deterioration could not be identified. Our study did not evaluate the proportion of patients whose HIV-1 status was known prior to tuberculosis diagnosis. In addition, we included patients initially assessed at the tuberculosis clinic, who subsequently presented to the hospital, and then deteriorated and died during their first hospital admission. Patients who presented to the hospital with fatal tuberculosis may not have been included in the study. A substantial proportion of patients were lost to follow-up (75 of 292 patients); reasons for this require further work. Loss to follow-up did not relate to the degree of immunosuppression of patients: In HIV-1 infected patients, the CD4+ counts of patients who were lost to follow-up were similar to the CD4+ counts of patients who were not lost to follow-up. Finally, this study was conducted at a tuberculosis clinic and a referral hospital that serve a large population with a high incidence of HIV-1-associated tuberculosis; thus, our findings may not be generalisable to other settings.

Conclusions

The two pandemics of HIV-1 and tuberculosis are inextricably intertwined in Africa. Reducing the extraordinary

burden of tuberculosis and clinical deterioration during antituberculosis treatment is a priority for resource-limited settings. Future prospective studies are required to determine (1) the optimal interval from antituberculosis treatment to ART initiation in profoundly immune-suppressed HIV-1 infected patients, and (2) whether ART initiation during antituberculosis treatment at higher CD4+ counts reduces the burden of clinical deterioration.

Disclaimer

The contents of this article are the responsibility of the authors and do not necessarily reflect the views of the US Agency for International Development or the US government.

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Authors' contributions

DJP, RJW and G. Meintjes were responsible for study conception. DJP, SM, RJW, G. Maartens, HM, VDA, HC, CM, and G. Meintjes were responsible for study design. DJP, SM, SS, JP, G. Meintjes were responsible for data acquisition. DJP, SM, RJW, FB, G. Maartens, HM, VDA, HC, CM, SS, JP, G. Meintjes were responsible for data interpretation. DJP compiled the first draft. SM, RJW, FB, G. Maartens, HM, VDA, HC, CM, SS, JP, and G. Meintjes critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Management of Patients With the Immune Reconstitution Inflammatory Syndrome

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A significant proportion of patients present with the immune reconstitution inflammatory syndrome (IRIS) after commencing antiretroviral therapy (ART). This syndrome is most frequently associated with infective causes. The lack of evidence-based treatment guidelines poses challenges in the management of these patients. Alternative causes for deterioration should be excluded, and optimization of treatment for the underlying opportunistic infection is essential. In addition, anti-inflammatory or immunomodulatory therapy may be considered, particularly in severe cases. Corticosteroids, the only treatment for which clinical trial data exist (for treating paradoxical tuberculosis-associated IRIS), are the treatment most frequently used in IRIS. Limited anecdotal reports of benefit exist for other agents, including NSAIDs, pentoxifylline, montelukast, thalidomide, and hydroxychloroquine. Therapeutic procedures (eg, aspiration of pus collections) play an important role in some patients. Interruption of ART may be considered in life-threatening forms of IRIS.

Introduction

The benefits of antiretroviral therapy (ART) in improving survival, reducing morbidity, and enhancing quality of life in HIV-infected patients have been widely demonstrated. However, during the early period of immune restoration on ART, a subset of patients (15%–25%) develops the immune reconstitution inflammatory syndrome (IRIS). IRIS manifests as clinical deterioration resulting from ART-augmented immune responses that cause inflammation in tissues directed at infective, or less frequently, noninfective antigens. Common forms are shown in Table 1. IRIS presents challenges to the clinician in terms of diagnosis, management, decisions as to whether to continue ART, and, if ART is to be continued, motivating patients to continue their therapy despite symptom deterioration. These challenges are further compounded by the lack of both a diagnostic test and an evidence base to guide management.

Infective IRIS can present as one of two forms: 1) paradoxical IRIS, in which patients on antimicrobial therapy for an infection experience clinical deterioration related to the infection after starting ART; or 2) unmasking IRIS, in which a previously present, but clinically undetected and therefore untreated infection, becomes apparent after starting ART, and the clinical presentation is unusually inflammatory in nature.

Infective IRIS results from an inappropriate or dysregulated immune response directed to pathogen-specific antigens. Most cases occur within the first 3 months of starting ART, coinciding with a rapid rise in peripheral blood CD4+ cells. Mycobacterial and fungal forms of IRIS usually present with features of a T helper 1 immune response, manifesting with granulomatous

inflammation, or suppuration. In contrast, CD8+ T cells are the dominant inflammatory cells found in IRIS related to viruses [1].

IRIS is a very heterogeneous condition. It may be associated with viral infections, bacteria, mycobacteria, fungi, protozoa, helminths, malignancies, autoimmune conditions, or other noninfective inflammatory conditions. It may be localized (eg, a single tuberculous lymph node enlarging) or involve several organ systems simultaneously. It may be mild and self-limiting within a few days, or persist for several years [2•,3•]. A minority of cases may be life-threatening or fatal, particularly when central nervous system (CNS) involvement or complications such as airway compromise, organ failure, or organ rupture occur. This heterogeneity makes it very difficult to propose generic treatment recommendations. Management usually requires an individualized approach.

We discuss the range of management options described, the settings in which they have been used, their benefits, and potential risks. We emphasize the importance of optimizing treatment of the underlying opportunistic infection. This review focuses exclusively on infective forms of IRIS.

Diagnosis of IRIS

Clinical deterioration is accelerated in the context of unmasking IRIS. For example, patients with unmasking pulmonary tuberculosis (TB) IRIS may present with a short history of respiratory symptoms and be acutely unwell with respiratory distress. Rapid diagnosis of the underlying opportunistic infection, to enable appropriate antimicrobial therapy, may be life-saving.

In patients with paradoxical IRIS there is no confirmatory diagnostic test, and the diagnosis is made by excluding alternative explanations for clinical deterioration. These include failure of treatment of the opportunistic infection (due to antimicrobial drug resistance, poor adherence, or drug malabsorption), an alternative infection, malignancy, or drug reaction. The diagnostic work-up will depend on the nature of the clinical presentation. For example, in patients with TB meningitis who develop recurrent meningitis after starting ART, differential diagnoses include antitubercular drug resistance, drug malabsorption, and other forms of meningitis. We advise caution when considering anti-inflammatory therapies, such as corticosteroids, for treating IRIS until alternative diagnoses have been excluded. If the diagnosis of IRIS is erroneous, such therapies may cause harm, particularly if the patient actually has treatment failure or another untreated infection.

Optimization of Treatment for the Underlying Infection

Treatment of the infection should be promptly initiated or optimized in unmasking and paradoxical IRIS, respectively. Appropriate treatment is essential to control replication and reduce antigen load of the infecting organism, the antigens of which are the stimuli that provoke and sustain IRIS. If anti-inflammatory therapy, with immunosuppressive effects, is used during suboptimal treatment of the underlying opportunistic infection, deterioration may well occur.

In patients with paradoxical IRIS, it is important to consider whether the treatment for the underlying infection is fully effective. An important consideration is antimicrobial drug resistance. Paradoxical TB-IRIS may occur in patients with undiagnosed multidrug-resistant TB, and it is clinically indistinguishable from paradoxical TB-IRIS that occurs in those with drug-susceptible TB [4•]. Where accessible, TB drug susceptibility testing should be performed in all patients with suspected paradoxical TB-IRIS. Treatment should be adjusted according to findings [4•]. Similarly, patients with cryptococcal meningitis who present with recurrent meningitis after starting ART may have undiagnosed fluconazole resistance [5•].

In mycobacterial avium complex (MAC) IRIS, combination anti-mycobacterial therapy should be guided by drug susceptibility testing, when possible. Intensification of MAC therapy (adding rifabutin, a quinolone, and/or an aminoglycoside to the macrolide and ethambutol combination) has been reported in certain patients with MAC-IRIS: those who do not experience a significant peripheral blood CD4+ cell response to ART, those without symptom improvement, and those who fail to eradicate active MAC infection [2•].

A proportion of patients with hepatitis B virus (HBV) co-infection who start ART develop an immune-mediated hepatitis flare due to IRIS. Controlling HBV replication prior to or at the time of ART initiation may prevent this or prevent recurrences when ART is reinitiated after interruption [6,7]. This may be achieved by pretreating patients with HBV-specific therapy or including drugs (preferably two) that are active against HBV in the ART regimen (tenofovir plus emtricitabine or lamivudine) [7]. HBV-IRIS has been reported, however, despite the inclusion of lamivudine in the ART regimens of two patients [6].

Cutaneous forms of IRIS generally respond to specific therapy for the associated condition and usually do not require anti-inflammatory therapy. For example, cases of herpes zoster occurring after ART typically respond to appropriate antiviral therapy. Topical corticosteroids have, however, been used (eg, in inflammatory folliculitis).

Anti-inflammatory and Immunomodulatory Therapy

In patients with more severe IRIS, in addition to optimizing treatment for the underlying pathogen, it may be necessary

to consider anti-inflammatory or immunomodulatory therapy to reduce inflammation and tissue damage, as well as alleviate symptoms.

Corticosteroids are not always effective [8•,9••], and they have potential side effects. This has led clinicians to use alternative agents in isolated cases reported in the literature or propose alternative agents for treating IRIS. We discuss agents that have been used with success in the management of IRIS; their mechanisms of action and potential risks are presented in Table 2. We also discuss tumor necrosis factor- α inhibitors because they have been proposed as a potential therapy. Many cases of IRIS are, however, mild and self-limiting and may require no anti-inflammatory therapy.

Corticosteroids

Corticosteroids are the most frequently used treatment in clinical practice, particularly in severe cases, and are the only treatment for which clinical trial data exist. Outside the context of IRIS, corticosteroids have been used as adjuvant therapy in a range of infectious diseases to reduce pathological host inflammatory responses. Mortality benefit has been demonstrated for bacterial meningitis, tuberculous meningitis, tuberculous pericarditis, severe typhoid fever, and pneumocystic pneumonia with moderate to severe hypoxemia. In a range of other infectious conditions, symptomatic benefit has been demonstrated [10••].

In paradoxical TB-IRIS, initial reports demonstrated symptom improvement with corticosteroid therapy [11]. We have recently reported results of a randomized placebo-controlled trial of prednisone for the treatment of paradoxical TB-IRIS. One hundred and ten patients were enrolled; those with life-threatening manifestations were excluded. Patients received prednisone or placebo at a dosage of 1.5 mg/kg for 2 weeks followed by 0.75 mg/kg for 2 weeks. We demonstrated a reduction in morbidity in the prednisone arm, in the form of a combined end point of days hospitalized plus outpatient therapeutic procedures. Significant benefit was also demonstrated in terms of symptom improvement. We found no excess of severe adverse events in the prednisone arm [12].

Corticosteroids have also been used to treat unmasking TB-IRIS and IRIS associated with MAC, leprosy, other nontuberculous mycobacterial infections, cryptococcosis, histoplasmosis, and *Pneumocystis jiroveci* [9••].

IRIS involving the CNS is relatively common [13•,14•] and is most frequently associated with cryptococcosis, TB, and JC virus (causing progressive multifocal leukoencephalopathy [PML]). Manifestations include enlarging space-occupying lesions, meningitis, encephalitis, myelitis, and radiculitis. Corticosteroid treatment has been most consistently used in these forms of IRIS because there is a potential risk for neurologic IRIS to cause death and long-term disability as a result of

inflammatory damage to brain or spinal cord tissue. In clinical trials, corticosteroids reduce mortality in TB meningitis, outside the context of IRIS. Evidence of benefit in neurologic IRIS is, however, anecdotal. We recently reported neurologic involvement in 23 of 190 (12%) patients with paradoxical TB-IRIS [13•]. Twenty of these 23 patients were treated with prednisone at a starting dosage of 1.5 mg/kg/day, and 18 showed initial clinical improvement. Documented 6-month survival among all 23 patients was 70%. Other authors have reported benefit with corticosteroids in paradoxical neurologic TB-IRIS [15,16] and unmasking neurologic TB-IRIS [17]. Recurrence of meningitis is the most common presentation of cryptococcal IRIS, and there are several reports of favorable responses to corticosteroids in these patients [18,19]. Corticosteroids have also been used with favorable response in other forms of CNS-IRIS, such as varicella zoster virus transverse myelitis [20] and a spinal mycobacterial mass lesion [21]. Variable responses to corticosteroids have been observed in patients with HIV encephalitis IRIS [22].

PML-IRIS may manifest as rapid neurologic deterioration in patients with known PML after commencing ART or with new onset of PML after commencing ART [14•]. In 57% of cases, PML-IRIS is associated with contrast enhancement on MRI [14•]. The role of corticosteroids in treating PML-IRIS is controversial. Outcomes varying from marked improvement to death have been demonstrated both with and without corticosteroid therapy [14•]. Although the inflammation associated with PML-IRIS may result in initial neurologic deterioration, it may ultimately be important in controlling JC virus replication. The presence of an effective cytotoxic T-lymphocyte response to JC virus has been associated with control of JC virus replication and improved prognosis [23]. It has been argued that attenuating this inflammatory response with corticosteroids may be deleterious [24]. Some cases of PML-IRIS have improved following transient worsening after starting ART [25], supporting this hypothesis. It has been proposed that corticosteroids should only be considered when there is significant cerebral edema and mass effect associated with PML-IRIS, because these features are imminently life-threatening [24,25]. In contrast, in a recent review of PML-IRIS, it was argued that there may be benefit from early and prolonged corticosteroid therapy. However, mortality was similar for those treated with and not treated with corticosteroids (42% vs 33%, respectively; $P = 0.73$) [14•]. An important consideration is that it is difficult to distinguish patients with deterioration due to IRIS from those with progressive PML due to JC virus infection that is poorly controlled by the immune response [26]. Steroid therapy for the latter scenario will very likely cause harm, particularly as no specific antiviral therapy for JC virus exists.

In cytomegalovirus immune recovery vitritis (CMV IRV), corticosteroid therapy is not always required but should be considered when vision is threatened [27]. Topical [27], repository [27,28], and rarely, oral [28] corticosteroids have been used with success in the treatment of CMV IRV.

In viral hepatitis-associated IRIS, corticosteroids may cause harm. Studies using corticosteroids in chronic hepatitis B outside the context of IRIS demonstrated increased mortality, increased hepatitis B replication, and worsening of biochemical parameters [10••]. A patient with HBV-IRIS died after receiving high-dose oral corticosteroids [6]. Their use for this form of IRIS appears ill-advised at present.

Different corticosteroid drugs have been used at a range of doses and durations in the management of IRIS. The American Thoracic Society guidelines suggest that severe TB-IRIS be treated with prednisone or methylprednisone at a starting dosage of 1 to 2 mg/kg/day, with gradual reduction after 1 to 2 weeks. Lesho [9••] suggests the use of prednisone 10 to 40 mg/day for moderate, and 1 to 2 mg/kg/day for severe IRIS associated with mycobacteria, fungi, and certain viruses. Most patients respond to a few weeks of corticosteroid therapy. Some patients experience symptom relapse when corticosteroids are tapered or discontinued, and require months of treatment [29•].

When considering corticosteroid therapy, the potential benefits should be weighed against the potential risks. Potential risks arise from further immune-suppression of an already immune-suppressed patient, and may result in increased susceptibility to infections or reactivation of latent infections. The risk of infections is likely to be higher with more prolonged corticosteroid therapy. Although previously used safely in HIV-infected patients [30], corticosteroids have been associated with the development of Kaposi's sarcoma [31], herpes simplex, and zoster virus flares [30]. Hypertension, hyperglycemia, and fluid retention are also documented in HIV-infected patients treated with corticosteroids. There are case reports of herpes zoster virus encephalitis [32] and life-threatening exacerbation of Kaposi's sarcoma [33•] in patients with IRIS treated with corticosteroids. Although infrequent, reactivation of CMV retinitis has been reported after the use of repository corticosteroids for CMV IRV [34]. In tropical/subtropical countries or patients originating from these countries, chronic *Strongyloides* infection may be present in up to 5%. Concerns have been raised that corticosteroids used to treat IRIS may predispose to *Strongyloides* hyperinfection syndrome in these patients (Boulware, personal communication).

An additional consideration is that most cases of IRIS are self-limiting. For a patient in whom the potential risks of corticosteroids are high (eg, a patient with Kaposi's sarcoma), it is probably safer to avoid corticosteroids and offer supportive and symptomatic therapy and counsel that

symptoms will improve, provided the IRIS is not life-threatening.

Figure 1 describes a patient with paradoxical TB-IRIS who responded well to corticosteroids but whose clinical course was complicated by the development of CMV retinitis that was likely a complication of the corticosteroid therapy.

NSAIDs

NSAIDs may provide symptom relief in patients with IRIS manifestations that are not severe [35]. Favorable responses have been reported in cryptococcal IRIS with lymphadenitis, MAC-IRIS, and paradoxical TB-IRIS. The most frequent side effects of nonselective cyclooxygenase inhibitors are gastric and intestinal ulceration. NSAIDs may worsen renal dysfunction in patients with HIV-associated nephropathy.

Thalidomide

Thalidomide is used in a range of inflammatory conditions, such as erythema nodosum leprosum and graft-versus-host disease. It has been used in isolated cases of IRIS: its use was associated with transient clinical improvement in a patient with TB lymphadenitis IRIS [36] and resolution of *Cryptococcus* lymphadenitis IRIS [18].

Common, dose-related side effects include somnolence, peripheral neuropathy, fatigue, constipation, rash, dizziness, tremor, mood changes, and headache. However, in a cohort of adult TB-HIV co-infected patients, thalidomide was well tolerated at a dosage of 200 mg daily [37]. Given its strong association with teratogenicity and potential toxicities (Table 2) [38], it is unlikely to be used as a treatment for IRIS other than in isolated cases that are severe and corticosteroid nonresponsive. Thalidomide has anecdotally been shown to result in marked clinical and radiographic improvement in pediatric and adult HIV-uninfected patients with intractable tuberculomas [39,40] and may have a role in the treatment of tuberculomas that enlarge with IRIS when response to corticosteroids is poor.

Pentoxifylline

Pentoxifylline has been used in various autoimmune diseases and infections, such as leprosy and leishmaniasis. Its use has been reported in two cases of IRIS: TB-IRIS manifesting as worsening hilar lymphadenopathy and radiographic pulmonary infiltrates, in which it was beneficial [41]; and *Mycobacterium celatum* pneumonitis IRIS, in which it was ineffective [42]. Pentoxifylline has few side effects and has been shown to be safe in a cohort of TB-HIV co-infected patients [43].

Hydroxychloroquine

Hydroxychloroquine is used in the treatment of various rheumatologic conditions. Hydroxychloroquine, in combination with methylprednisolone and interruption of

ART, was successfully used to treat a case of cryptococcal meningitis IRIS [44]. Short-term hydroxychloroquine is generally well tolerated at a dosage of 400 mg per day.

Leukotriene antagonists

Montelukast and zafirlukast are leukotriene antagonists used in the treatment of asthma. There are reports of montelukast being used to treat IRIS in three patients. Two patients who initially had a poor response to corticosteroids responded rapidly to montelukast—one patient with urticarial rash and fever ascribed to secondary syphilis IRIS, and another patient with TB-IRIS with lymphadenitis and fever [8•]. Another patient with urticarial vasculitis associated with the re-introduction of ART responded to montelukast [45].

Peripheral blood monocytes and neutrophils and alveolar macrophages from HIV-infected patients show reduced leukotriene synthesis [46]. It has been proposed that IRIS may be partly due to an overexuberant reconstitution of leukotriene activity [8•]. Montelukast is well tolerated, with side effects comparable to placebo. Increased susceptibility to infections has not been observed in studies in which montelukast was administered to adult asthma patients [47]. Because the drug is partly metabolized through cytochrome P450 3A4 [47], plasma levels will be affected by certain antiretrovirals and rifampicin.

Given that montelukast has few side effects and does not appear to be associated with an increased risk of infection, it warrants further investigation, as an alternative to corticosteroids. Furthermore, the role of leukotrienes in the pathogenesis of IRIS needs to be explored to provide the rationale for taking this agent into clinical trials.

Tumor necrosis factor- α inhibitors

None of the agents (infliximab, etanercept, adalimumab, or certolizumab pegol) in this class has been used to treat IRIS. However, it has been proposed that they may be effective, given that they profoundly inhibit cellular responses to infection. They are used in the treatment of rheumatoid arthritis, spondyloarthropathies, and Crohn's disease. Infliximab was used in a patient who was not on ART with a debilitating TB paradoxical reaction manifesting with multiple tuberculomas and cerebral edema that was resistant to corticosteroids and cyclophosphamide. Three doses of infliximab at monthly intervals resulted in good clinical and radiographic response [48•].

Infliximab and the other agents in this class are expensive. Infliximab can only be administered intravenously, and a range of serious adverse events have been reported, including increased risk of infections (mycobacterial, fungal, viral, and bacterial), anaphylactoid infusion reactions with infliximab, demyelinating CNS disease, malignancy, and induction of autoimmunity. The risk of infections is particularly concerning in HIV-infected

patients starting ART, many of whom are already profoundly immune-suppressed. In addition, these drugs have a long half-life (7–12 days for infliximab), meaning that if infections occur, the drug effect cannot easily be withdrawn. Given these serious risks, these agents only warrant consideration for the treatment of life-threatening IRIS (particularly TB-IRIS of the CNS) that is unresponsive to corticosteroids and after thorough work-up to exclude latent infections.

Other Medical Therapies

Other immunomodulatory therapies used to treat mycobacterial IRIS include granulocyte colony-stimulating factor [2•,49], interleukin-2 [15,49,50], and granulocyte-macrophage colony-stimulating factor [50]. Intravenous immunoglobulin was used to treat parvovirus B19 IRIS encephalitis, and acetazolamide has been used to treat increased intraocular pressure in CMV IRV [27].

Pain and fever are common symptoms that may respond to simple analgesia and antipyretic drugs. Opiates may be required for severe pain. Parenteral fluids, nutritional support, and antiemetic drugs may be required in cases of IRIS involving abdominal organs or the gastrointestinal tract. Seizures may complicate CNS IRIS [13•,18] and require anticonvulsants.

Therapeutic Procedures

Suppuration of lymph nodes and formation of cold abscesses are common manifestations of IRIS associated with mycobacterial and cryptococcal disease. To provide symptom relief, surgical drainage, needle aspiration, or lymphadenectomy have been performed in these patients [15]. However, surgical procedures on such pus collections may be complicated by chronic sinus formation that may be large and disfiguring. Rather, we advise that such nodes and abscesses are drained with a wide-bore needle, which, in our experience, reduces the risk of large sinus formation. Repeated aspiration of pus collections may be necessary in TB-IRIS. In a series of 25 patients with TB-IRIS, six had surgical drainage of nodal or soft tissue abscesses and 11 had needle aspirations performed, four of them on multiple occasions (up to 108 aspirations) [3•].

Surgical intervention has been required in a number of cases to treat the complications of IRIS. These surgical procedures were indicated for bowel perforation due to TB IRIS [19], partial bowel obstruction due to cryptosporidiosis IRIS, spinal abscess associated with cryptococcal IRIS [18], and cases in which TB-IRIS resulted in splenic rupture. Cases of MAC-IRIS associated with endobronchial, spinal, cerebral, and ocular disease, as well as pyomyositis and septic arthritis, have also required surgery [11]. Vitrectomy procedures have been performed in patients with CMV IRV [51].

Increased intracranial pressure complicates cryptococcal meningitis IRIS in most patients. Increased cerebrospinal fluid (CSF) pressure in cryptococcal meningitis is associated with increased morbidity and mortality unless managed aggressively [52•]. Daily lumbar punctures should be performed for patients with opening pressure more than 25 cm H₂O, with the removal of up to 20 to 30 mL of CSF to reduce opening pressure to less than 20 cm H₂O or by 50%. This should be repeated until the opening pressure is normal for several days. Lumbar drains and ventricular shunts have occasionally been required to reduce increased CSF pressure.

Interruption of ART

It remains controversial as to whether ART should be interrupted in patients presenting with IRIS. Although ART can usually be continued safely in most cases of IRIS, some authors suggest interrupting ART when IRIS is life-threatening or unresponsive to corticosteroids [1,11]. There are reports of response to this strategy. One situation in which we interrupt ART is in patients who present with neurologic manifestations of IRIS and a depressed level of consciousness. Interrupting ART, especially in patients who are severely immune-suppressed, may increase vulnerability to other opportunistic infections and predispose to ART drug resistance. Furthermore, IRIS may recur when ART is reinitiated.

Viral hepatitis IRIS is difficult to distinguish from drug-induced liver injury. When hepatitis is severe, even if IRIS is thought to be the cause, it may be necessary to interrupt ART and other potentially hepatotoxic drugs, given that it is difficult to exclude drug-induced injury as the etiology. In less severe cases it may be possible to continue ART and monitor liver function tests closely.

Conclusions

The diagnostic evaluation of a patient presenting with IRIS requires the exclusion of other causes for deterioration. In our experience, the most important diagnosis to exclude is antimicrobial drug resistance of the underlying opportunistic infection [4•,5•]. Treatment of the underlying opportunistic infection should be initiated or optimized. In mild cases, no additional therapy is required. Corticosteroids should be considered in cases with significant symptoms and certainly when IRIS is life-threatening. Clinical trials evidence exists for corticosteroid use in paradoxical TB-IRIS. There is also a substantial body of anecdotal evidence for their use in other forms of IRIS, particularly mycobacterial, fungal, and certain forms of viral IRIS [9••]. Corticosteroids may have a role in PML-IRIS that is imminently life-threatening, but caution has been advised in less severe cases. Corticosteroids should not be prescribed in IRIS associated with viral hepatitis. In most instances, ART should be continued,

apart from when IRIS is life-threatening and not responding to corticosteroids. Symptomatic therapy and NSAIDs have also been used. There are currently insufficient data to recommend any of the other anti-inflammatory or immunomodulatory therapies discussed in this review; further research is required. It is important to appropriately counsel patients with symptom deterioration due to IRIS in order to ensure continued ART adherence.

Ongoing research regarding the immune pathogenesis of IRIS will likely direct future rational therapeutic approaches and clinical trials.

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Disclosure

No potential conflicts of interest relevant to this article were reported.

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- Of importance
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Figure 1. Paradoxical pulmonary and pleural tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS) treated with prednisone. A 43-year-old HIV-infected woman was diagnosed with disseminated tuberculosis. An aspirate from a cervical node cultured *Mycobacterium tuberculosis* (susceptible to rifampicin [R] and isoniazid [H]), and her chest radiograph showed a bilateral reticulonodular infiltrate with large hilar adenopathy (A). She commenced antitubercular treatment—H, R, pyrazinamide (Z), and ethambutol (E). Her cough resolved, and her night sweats and appetite improved. Chest radiography also improved (B). Her CD4 count nadir was 20 cells/μL. Ten weeks after starting antitubercular treatment she initiated combination antiretroviral therapy (ART)—stavudine (30 mg twice daily), lamivudine (150 mg twice daily), and efavirenz (600 mg at night). Eight days later, she developed shortness of breath and recurrence of her cough. Her CD4 count had increased to 64 cells/μL. Chest radiography at TB-IRIS showed a new right pleural effusion and recurrence of pulmonary infiltrate (C). We drained 750 mL of straw-colored pleural fluid, which did not culture mycobacteria. We continued HRZE and ART, and prescribed prednisone, 70 mg daily (1.5 mg/kg/day) for 4 weeks. Her cough and night sweats resolved. Chest radiography showed dramatic improvement (D). She, however, developed cytomegalovirus retinitis of her left eye. Prednisone was stopped, and she was treated with intravitreal ganciclovir, with improvement in vision. Six months after ART initiation, her CD4 count was 263 cells/μL, and HIV-1 viral load was undetectable.

Neuroradiological features of the tuberculosis-associated immune reconstitution inflammatory syndrome

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SUMMARY

SETTING: Paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is an important complication in human immunodeficiency virus type I (HIV-1) infected tuberculosis (TB) patients who start combination antiretroviral treatment (ART). Neurological manifestations occur in more than 10% of TB-IRIS cases. Apart from a few case reports, the radiological features of neurological TB-IRIS have not been described.

OBJECTIVE: To describe the neuroradiological findings of patients with paradoxical neurological TB-IRIS.

DESIGN: Computed tomography (CT; $n = 13$) and magnetic resonance imaging ($n = 3$) findings of 16 patients were reviewed.

RESULTS: IRIS manifestations included meningitis ($n = 4$), intracranial space occupying lesions (SOLs, presumed tuberculomas; $n = 5$), meningitis and SOLs ($n = 5$),

radiculomyelitis ($n = 1$) and spondylitis ($n = 1$). In patients with tuberculoma IRIS, we observed a high prevalence of 1) low density lesions on non-contrast-enhanced CT (all lesions), 2) multiple lesions (in 5/10 patients) and 3) perilesional oedema (17/22 lesions). In patients with meningitis, meningeal enhancement ($n = 2$) and hydrocephalus ($n = 1$) were infrequently observed.

CONCLUSION: This is the first substantial series to describe the radiological features of paradoxical neurological TB-IRIS. Compared to published radiological findings of tuberculomas in HIV-1-infected patients (not receiving ART), an increased inflammatory response is suggested in tuberculoma IRIS. However, this was not observed in patients with TB meningitis IRIS.

KEY WORDS: immune reconstitution inflammatory syndrome (IRIS); neuroradiology; HIV; tuberculoma

DISEASE due to *Mycobacterium tuberculosis* is the main cause of morbidity and death in human immunodeficiency virus type 1 (HIV-1) infected persons worldwide. The disease is disproportionately prevalent in resource-limited countries.¹ The increased availability of combination antiretroviral treatment (ART) in these countries has played a significant role in reducing morbidity and mortality associated with HIV-1/*M. tuberculosis* co-infection.² Despite the benefit of ART in improving clinical outcome, patients may deteriorate clinically and/or radiologically after commencing ART due to the immune reconstitution inflammatory syndrome (IRIS).^{3,4} IRIS is associated with a wide spectrum of infectious and non-infectious diseases, and mycobacteria are frequently implicated.³ Tuberculosis-associated IRIS (TB-IRIS) may present in one of two recognised ways: first as paradoxical worsening of symptoms after commencement of ART, and second, as an 'unmasking' syndrome.⁴ Unmasking TB-IRIS occurs when HIV-1-infected patients who

have unrecognised tuberculosis (TB) start ART and subsequently develop clinical manifestations of TB that may have a prominent inflammatory component. Lymph node enlargement and pulmonary involvement are common manifestations of TB-IRIS; clinical and radiological features are well described.^{3–6} Neurological TB-IRIS, although accounting for over 10% of paradoxical TB-IRIS cases in a hospital setting,⁷ is less well documented; only one clinical case series⁷ and a handful of radiological case reports exist.^{5,6,8–14} Here we describe the radiological features of paradoxical neurological TB-IRIS.

STUDY POPULATION AND METHODS

We have previously reported our patient cohort in a prospective clinical case series of paradoxical neurological TB-IRIS.⁷ We identified 23 cases of neurological TB-IRIS from a cohort of 279 adult paradoxical TB-IRIS suspects at GF Jooste Hospital, a district

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public hospital in Cape Town, South Africa. Of these 23 patients, 16 patients' neuroradiological findings are reported in this paper. Data published included demographic characteristics, TB investigations, clinical examination and laboratory findings as well as management and outcome.⁷ Microbiologically confirmed tuberculosis was diagnosed when acid-fast bacilli (AFB) were visualised or *M. tuberculosis* was cultured from a clinical specimen. Microbiologically non-confirmed tuberculosis was diagnosed according to World Health Organization (WHO) guidelines for HIV-1-infected patients with smear-negative or extra-pulmonary TB.¹⁵ TB meningitis (TBM) was diagnosed when clinical and laboratory findings were consistent with the diagnosis. Paradoxical neurological TB-IRIS was defined according to a modified published consensus clinical case definition (Table 1).^{4,7} All cases were regarded as probable, because a confirmatory laboratory test for TB-IRIS is not available. In all patients, ART consisted of two nucleoside- and one non-nucleoside reverse transcriptase inhibitor.

Computed tomography (CT) of the head was performed at GF Jooste Hospital or at Groote Schuur Hospital (GSH), a tertiary referral hospital in Cape Town. Scanners utilised at these facilities were Philips Brilliance Air 6 Multi slice scanner (Philips, Amsterdam, The Netherlands) and Siemens Somatom Balance Single slice spiral scanner (Siemens, Berlin, Germany), respectively. In each case, 5-mm contiguous axial scans were obtained from skull base to vertex before and/or after intravenous administration of 50 ml iodinated contrast medium, Ultravist-300®

Table 1 Case definition for paradoxical neurological TB-IRIS

The case definition includes three components:

Antecedent requirements

Both of the two following requirements must be met:

- Diagnosis of TB: the TB diagnosis was made before starting combination ART and this should fulfil World Health Organization criteria for diagnosis of smear-positive PTB, smear-negative PTB or extra-pulmonary TB.
- Initial response to TB treatment: the patient's condition should have stabilised or improved on appropriate anti-tuberculosis treatment prior to ART initiation, e.g., cessation of night sweats, fevers, cough, weight loss. (Note: this does not apply to patients starting ART within 2 weeks of starting anti-tuberculosis treatment, as insufficient time may have elapsed for a clinical response to be observed).

Clinical criteria

The onset of neurological TB-IRIS manifestations should be within 3 months of ART initiation, re-initiation, or regimen change due to treatment failure. These include:

- New or worsening TB meningitis
- New or worsening intracerebral space occupying lesion, likely tuberculoma
- New or worsening spinal TB

Alternative explanations for clinical deterioration must be excluded, if possible:

- Failure of anti-tuberculosis treatment due to TB drug resistance
- Poor adherence to anti-tuberculosis treatment
- Another opportunistic infection or neoplasm
- Drug toxicity or reaction

TB = tuberculosis; ART = antiretroviral treatment; PTB = pulmonary TB.

(Bayer Schering Pharma, Berlin, Germany). Magnetic resonance imaging (MRI) was performed on a Siemens Magnetom Symphony 1.5 Tesla scanner using standard multiplanar T1 and T2 spin-echo sequences with-out and with gadolinium administration (Magnevist®, Bayer Schering). CT and MRI images were retrospectively evaluated by an experienced neuroradiologist and a neuroradiologist in training. Both were blinded to clinical information.

CT brain scans were evaluated for the presence or absence of 1) brain atrophy, 2) hydrocephalus, 3) meningeal enhancement, 4) infarction/white matter oedema and 5) parenchymal space occupying lesions (SOLs). When SOLs were visualised, their location, size, number and enhancement pattern were recorded. Where available, follow-up imaging studies were reviewed and compared with scans at presentation. Although neuro-imaging was obtained in 21 of the 23 patients at the time of neurological TB-IRIS, only 16 patients' scans were available later for blinded review. These 16 patients comprise the study population.

The Research Ethics Committee of the University of Cape Town approved the study (REC494/2008).

RESULTS

The neurological presentations of paradoxical TB-IRIS included meningitis ($n = 4$), intracranial SOLs: presumed tuberculomas ($n = 5$), both meningitis and SOLs ($n = 5$) and spinal TB ($n = 2$). The clinical presentation and laboratory data are summarised in Table 2. Of the 16 patients, 10 were female. Age ranged from 23 to 44 years (median 30). The median CD4⁺ T-lymphocyte count before starting ART was 70 cells/ μ l (interquartile range [IQR] 19–135), and the median rise in CD4⁺ T-lymphocyte count within the first year of starting ART was 284 cells/ μ l (IQR 103–368). The onset of neurological TB-IRIS symptoms occurred 15 days (median IQR 5–31) after ART initiation. The median duration from initiation of anti-tuberculosis treatment to ART was 56 days (IQR 35–105).

Good adherence to both anti-tuberculosis treatment and ART was documented in all but one patient. This patient initially defaulted from anti-tuberculosis treatment, but was subsequently adherent to in-patient treatment for 3 months before starting ART. Microbiological confirmation of TB was obtained in 12 of the 16 patients. In three patients, lumbar puncture was contraindicated because of intracerebral or intraspinal mass effect. Alternative diagnoses were excluded, as previously published.⁷ In all patients who presented with meningitis ($n = 9$), cryptococcal and bacterial meningitis were excluded. In patients with meningitis and/or SOLs ($n = 14$), syphilis serological tests were negative in 11, positive in one, and not performed in two patients. Case 9, with a positive syphilis serological test, and Case 8, in whom the

Table 2 Baseline characteristics, TB–HIV-1 data, and details of neurological symptoms for 16 patients* with paradoxical neurological TB–IRIS†

Case	Sex	Age, years	Previous TB	Site of TB before ART	Initial TB result (site)	CD4 pre-ART	CD4 post-ART	Time from ART initiation to CD4 count (days)	Neurological symptoms and signs at TB–IRIS event
1	Female	37	No	Disseminated	No microbiological confirmation	17	—	—	Headache, left arm and leg seizures, confusion
2	Female	43	No	Meningitis, SOL	AFB visualised (CSF)	3	—	—	Headache, neck stiffness, confusion
3	Female	30	No	Meningitis	No microbiological confirmation	95	423	187	Headache, neck stiffness, generalised tonic clonic seizures
4	Female	28	Yes	Disseminated	<i>M. tuberculosis</i> cultured (sputum)	33	—	—	Seizures, confusion
5	Female	36	No	Disseminated	AFB visualised (sputum)	50	—	—	Headache, neck stiffness, vomiting, agitation
6	Male	44	No	Disseminated	<i>M. tuberculosis</i> cultured (node)	134	502	224	Headache, focal seizures of right arm
7	Male	35	Yes	Pleural	<i>M. tuberculosis</i> cultured (sputum)	139	423	151	Seizures, left hemiparesis
8	Female	24	No	Disseminated	<i>M. tuberculosis</i> cultured (node)	12	6	23	Generalised tonic clonic seizures, post-ictal confusion
9	Male	43	No	Nodal	AFB visualised (node + sputum)	89	260	178	Headache, cognitive impairment, left arm weakness
10	Female	23	Yes	Disseminated	<i>M. tuberculosis</i> cultured (CSF)	254	1043	214	Headache, generalised tonic clonic seizures
11	Female	30	Yes	Abdominal nodes	No microbiological confirmation	19	104	157	Headache, confusion, neck stiffness, photophobia, vomiting
12	Male	27	Yes	Disseminated	AFB visualised (sputum)	48	—	—	Headache, neck stiffness
13	Male	37	Yes	Disseminated	AFB visualised (sputum)	8	111	221	Neck stiffness, confusion, generalised tonic clonic seizures
14	Male	30	No	Meningitis	No microbiological confirmation	158	—	—	Headache, vomiting, neck stiffness
15	Female	31	No	Disseminated	<i>M. tuberculosis</i> cultured (sputum)	99	703	173	Right leg weakness, lower back pain radiating to the right hip
16	Female	26	Yes	Disseminated	<i>M. tuberculosis</i> cultured (sputum)	137	—	—	Headache, lower limb weakness, incontinence of urine + faeces
%	Male: 38		Yes: 44						
Median		30				70	423	178	
IQR		28–37				19–135	111–502	157–214	

* All patients received d4T/3TC/EFV ART except patient 13, who received AZT/3TC/EFV, and patient 2, who received d4T/3TC/NVP.

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TB = tuberculosis; HIV-1 = human immunodeficiency virus type 1; TB–IRIS = TB immune reconstitution inflammatory syndrome; ART = combination antiretroviral treatment; SOL = space occupying lesion; AFB = acid-fast bacilli; CSF = cerebrospinal fluid; IQR = interquartile range; AZT = zidovudine 300 mg twice daily; 3TC = lamivudine 150 mg twice daily; EFV = efavirenz 600 mg every night; d4T = stavudine 30 mg twice daily; NVP = nevirapine 200 mg twice daily.

test was not performed, had microbiological evidence of extra-central nervous system (CNS) TB at the time of neurological deterioration. Both patients in whom syphilis serological tests were not performed (Cases 3 and 8) improved without concomitant treatment for neurosyphilis.

Immunoglobulin G serological assays for *Toxoplasma* species was performed in 9 of 10 patients with SOLs. Although the test was positive in six patients, all six were receiving toxoplasmosis chemoprophylaxis prior to neurological deterioration. In addition, all six patients had radiological features of TB outside of the nervous system. The one patient who did not

have *Toxoplasma* species serology performed (Case 3) was alive 6 months after initial assessment without receiving treatment with therapeutic trimethoprim-sulfamethoxazole (320 mg/1600 mg twice daily) for possible toxoplasmosis. The median duration from neurological symptom onset to imaging was 18 days (IQR 9–43). Before imaging, nine patients received corticosteroid treatment for a median duration of 5 days (IQR 4–15).

Brain imaging findings

CT scans of the head were performed in 13 patients and MRI was performed in one patient (see Tables 3

Table 3 CT and MRI findings in 10 patients with SOLs with/without meningitis

Case	Disease category	Duration of cortico-steroids, days*	Imaging	Meningeal enhance-ment	Cerebral atrophy	Hydro-cephalus	Infarction	Initial scan: SOL + oedema				Follow-up scan†		
								Number of SOL	Position of SOL	Size, mm	Densities	Oedema	SOL	Oedema
1	SOL + meningitis	—	NECT CECT	No	Yes	No	No	3	Lobar Lobar DWM	5 25 <5	LD/HCE LD/NCE LD/NCE	Yes No No	↓ size Resolved No change	Oedema No change No change
2	SOL + meningitis	2	NECT CECT	No	—	No	No	3	DWM BG BG	20 10 20	LD/RCE LD/RCE LD/RCE	Yes Yes Yes	—	—
3	SOL + meningitis	4	NECT CECT	Yes, focal	No	No	No	1	Lobar	15	LD/ICE	Yes	—	—
4	SOL + meningitis	—	MRI + Gadolinium	No	No	No	No	1	Lobar	20	LD/RCE	Yes	—	—
5	SOL + meningitis	45	CECT	No	No	No	Yes, white matter	1	Lobar	10	ICE‡	Yes	—	—
6	SOL	—	NECT CECT	No	Yes	No	No	4	Lobar Lobar Lobar Lobar	14 14 15 6	LD/HCE LD/RCE LD/HCE LD/HCE	Yes Yes Yes Yes	↓ size ↑ size ↑ size Resolved	Reduced Increased No change Resolved
7	SOL	—	NECT CECT	No	Yes	No	No	1	Lobar	10	LD/HCE	Yes	—	—
8	SOL	—	NECT CECT	No	Yes	No	No	4	Lobar Lobar Lobar Lobar	12 10 10 <5	LD/RCE LD/HCE LD/RCE LD/HCE	Yes Yes Yes Yes	—	—
9	SOL	15	CECT	No	Yes	Yes	No	1	BG	12	ICE‡	Yes	—	—
10	SOL	5	CECT	Yes, focal	Yes	No	No	3	Lobar Lobar Lobar	20 10 7	ICE‡ ICE‡ ICE‡	No No No	—	—

* Duration of corticosteroid treatment at time of first imaging.

† Time interval between initial scan and follow up scan: Patient 1, 17 days; Patient 6, 341 days (last scan performed).

‡ Presumed enhancement; no NECT performed.

CT = computed tomography; MRI = magnetic resonance imaging; SOL = space occupying lesion; NECT = non-contrast-enhanced CT; CECT = contrast-enhanced CT; LD = hypodense/hypointense (densities pre-contrast); HCE = homogeneous enhancement (enhancement pattern); NCE = no contrast enhancement (enhancement pattern); DWM = deep white matter (position of lesions); RCE = rim contrast enhancement (enhancement pattern); BG = basal ganglia (position of lesions); ICE = inhomogeneous enhancement (enhancement pattern).

Table 4 CT findings in four patients with meningitis only

Case	Age/sex	Duration of corticosteroids, days*	Imaging	Meningeal enhancement	Hydrocephalus	Infarction	Atrophy
11	29/female	—	NECT + CECT	No	No	No	Yes
12	27/male	6	CECT	Yes, basal	Yes	Yes, MCA	Yes
13	37/male	—	CECT	No	No	No	Yes
14	29/male	2	CECT	No	No	No	Yes

*Duration of corticosteroid treatment at time of imaging.

CT = computed tomography; NECT = non-contrast enhanced CT; CECT = contrast enhanced CT; MCA = middle cerebral artery.

and 4). In the majority (10/14) of the patients, significant age-inappropriate generalised atrophy was noted. Ten patients had a total of 22 SOLs, five solitary and five multiple (see Figures 1 and 2 for illustrative cases). All lesions were supratentorial; 17 had perilesional oedema but only two had significant mass effect.



Figure 1 Illustrative case of paradoxical tuberculoma IRIS (Patient 9). A 43-year-old HIV-1-infected male was diagnosed with AFB smear-positive lymph node TB. His CD4+ T-lymphocyte count was 89 cells/ μ l, and he was receiving trimethoprim-sulfamethoxazole (160/800 mg daily) as primary prophylaxis. He showed clinical improvement on anti-tuberculosis treatment and was started on combination ART (stavudine, lamivudine, efavirenz) 50 days after anti-tuberculosis treatment was initiated. Fourteen days after starting ART, he developed headache, cognitive impairment and left upper limb weakness. One month later, he also developed an enlarged, fluctuant left supraclavicular lymph node. Fine-needle aspiration of lymph node revealed AFB, but TB culture was negative. Axial images of contrast enhanced computed tomography of the brain performed 56 days after neurological symptom onset showed generalised cerebral atrophy and communicating hydrocephalus. A 12 mm mixed hypodense and hyperdense lesion with surrounding oedema was present in the right thalamus. Lumbar puncture was deferred because of intracerebral mass effect. He was continued on ART, anti-tuberculosis treatment and prophylactic trimethoprim-sulfamethoxazole. Prednisone, which he received for a total duration of 69 days, was started at a dose of 1.5 mg/kg. His weakness resolved, and he was alive after 180 days of starting ART. Six months after ART was commenced his CD4+ T-lymphocyte count was 260 cells/ μ l and his HIV viral load was undetectable. IRIS = immune reconstitution inflammatory syndrome; HIV = human immunodeficiency virus; AFB = acid-fast bacilli; TB = tuberculosis; ART = antiretroviral treatment.

Three lesions were <6 mm, 8 between 6 and 10 mm, 10 between 11 and 20 mm and only one lesion was >20 mm. The median size was 11 mm (IQR 10–15). Pre-contrast, all lesions had lower attenuation than surrounding brain. Contrast enhancement was present in 20 lesions. Obstructive hydrocephalus was observed in one patient who had visible basal meningeal enhancement as well as a subacute middle cerebral perforator infarct. Communicating hydrocephalus was observed in one (Patient 9). Small areas of focal leptomeningeal enhancement were present in an additional two patients. Another patient had an infarct in the white matter of the centrum semiovale.

Spinal imaging findings

MRI of the spine was performed in two patients presenting with myeloradiculopathic symptoms. In one (Patient 15), the features were consistent with tuberculous spondylitis, with an epidural collection causing thecal sac compression at L4. In the other (Patient 16), we observed features of adhesive arachnoiditis with extensive leptomeningeal enhancement throughout the spinal canal. This was associated with diffuse cord oedema, presumably the result of meningo-vascular compromise. In addition, basal meningeal enhancement of the brain was observed in this case (see Figure 3).

DISCUSSION

We report the radiological findings of 16 patients whose clinical presentation met diagnostic criteria for paradoxical neurological TB-IRIS. These fall into five groups based on a combination of clinical, cerebrospinal fluid (CSF) and radiological findings: 1) SOLs without meningitis ($n = 5$), 2) meningitis without SOLs ($n = 4$), 3) meningitis and SOLs ($n = 5$), 4) radiculomyelitis ($n = 1$) and 5) spondylitis ($n = 1$).

Parenchymal space occupying lesions

The development of new or expanding intracranial tuberculomas as a manifestation of neurological TB-IRIS has been described previously.^{5–9,14} Although parenchymal tuberculomas are commonly reported in HIV-1-infected patients,¹⁶ some studies suggest that multiple tuberculomas occur less frequently than in non-HIV-1-infected patients. Brain imaging findings



Figure 2 Illustrative case of paradoxical tuberculoma IRIS (Patient 6). A 44-year-old HIV-1-infected male was diagnosed with pulmonary and culture-positive lymph node TB. His CD4⁺ T-lymphocyte count was 134 cells/ μ l, and he was receiving trimethoprim-sulfamethoxazole (160/800 mg daily) as primary prophylaxis. He improved on anti-tuberculosis treatment and was started on combination ART (stavudine, lamivudine, efavirenz) 27 days after commencing TB treatment. Two days after starting ART, he developed painful bilateral cervical lymphadenopathy. Lymph node aspirate cultured *M. tuberculosis* susceptible to rifampicin and isoniazid. Nineteen days after ART initiation the patient developed headache and focal seizures of his right arm. Neurological examination revealed an expressive aphasia which had been present since a left carotid injury resulting in a left MCA infarct several years before. Lumbar puncture performed was normal, and *Toxoplasma gondii* serum serology negative. Axial CECT (A) brain images performed 18 days after neurological symptom onset showed generalised cerebral atrophy, an old left MCA infarct, and multiple bilateral contrast enhancing lesions with surrounding oedema. The patient was diagnosed with neurological TB-IRIS and commenced on prednisone 1.5 mg/kg/day. Initially ART was continued. Serial brain imaging was performed over the next 11 months. Follow-up CECT performed 3 months later (B) showed progression in size, loculation and surrounding oedema of lesions. The patient was subsequently treated with therapeutic trimethoprim-sulfamethoxazole (320/1600 mg twice daily) for possible *T. gondii* encephalitis, with no radiological response 2 and 4 weeks later. ART was stopped at this time point but restarted 3 months later. Three repeat scans performed over the next 6 months showed radiological reduction in lesion size and surrounding oedema. (C shows the last CECT performed, 11 months after the first). The patient received prednisone for a total duration of 347 days. He was alive 180 days after starting ART, but subsequently died after defaulting from anti-tuberculosis treatment. His CD4⁺ T-lymphocyte count 7 months after starting ART was 502 cells/ μ l. IRIS = immune reconstitution inflammatory syndrome; HIV = human immunodeficiency virus; TB = tuberculosis; ART = antiretroviral treatment; MCA = middle cerebral artery; CECT = contrast-enhanced computed tomography.

for 100 patients with intracranial tuberculoma from a low HIV-1 prevalence population¹⁷ showed multiple lesions in 69% of patients. A cohort of predominantly HIV-1-infected patients not on ART showed multiple lesions in only 33% of cases.¹⁸ In our study, multiple lesions were present in 50% of patients. Of interest is our finding that all SOLs were of low density on non-contrast-enhanced CT (NECT). A study in a low HIV prevalence population¹⁷ reported low density on NECT in only 3% of tuberculous lesions. In our cohort, 17/22 (77%) lesions had a variable degree of oedema, with significant mass effect in two. In an ART-naïve HIV-1-infected TBM cohort, Thonell et al. found perilesional oedema in less than 50% of tuberculomas.¹⁸ The high prevalence of multiple lesions and surrounding oedema may reflect increased CNS inflammation in the context of neurological TB-IRIS. Radiological features consistent with increased inflammation have been observed in neurological IRIS associated with other pathogens, including *Polyomavirus* JC and *Cryptococcus neoformans*.¹⁹ TB abscess formation has been reported as a rare complication of CNS TB.²⁰ TB abscesses are usually solitary, multiloculated, thin-walled enhancing lesions, often >3 cm in diameter.²⁰ Although previously described as a neurological TB-IRIS manifestation,^{9,11} none of the

parenchymal lesions documented in our patients met the radiological criteria for intracerebral TB abscess.

The imaging appearance of intracranial tuberculomas is non-specific, toxoplasmosis being the most frequent differential diagnosis in HIV-1-infected persons not on ART. In a brain biopsy study from South Africa conducted in HIV-1-infected patients,²¹ a solitary parenchymal lesion was due to toxoplasmosis in 39% of cases. Other confirmed diagnoses in this study included brain abscess (16%), tuberculoma (11%), CNS cryptococcal infection (5%) and infarct (3%). For a quarter of patients, a specific histological diagnosis could not be obtained. No cases of primary CNS lymphoma, an additional differential diagnosis, were reported in this study. Dual infection of the CNS with *M. tuberculosis* and *Toxoplasma gondii* has also been reported.^{22,23} In the context of ART and trimethoprim-sulfamethoxazole prophylaxis, the diagnosis of 'unmasking' toxoplasmosis becomes less likely, but should still be considered.²⁴ In the absence of invasive brain biopsy, the diagnosis of solitary or multiple parenchymal lesions remains presumptive based on a combination of imaging findings together with CSF findings, ancillary tests (toxoplasmosis serology, CD4⁺ T-lymphocyte count, evidence for TB or TB-IRIS elsewhere) and response to treatment.⁷

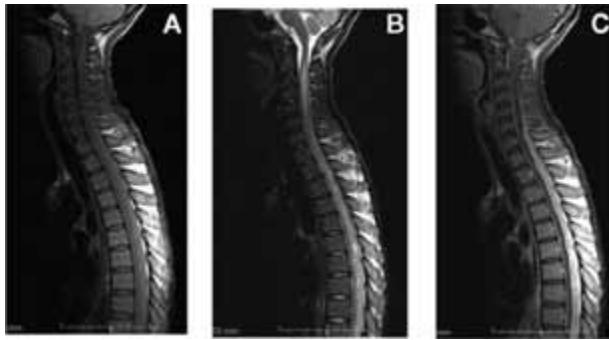


Figure 3 Illustrative case of paradoxical TB radiculomyelitis IRIS (Patient 16). A 26-year-old HIV-1-infected female was diagnosed with culture-positive pulmonary TB (susceptible to rifampicin and isoniazid) and TB meningitis. Her CD4+ T-lymphocyte count was 137 cells/ μ l. She showed clinical improvement on anti-tuberculosis treatment, and combination ART (stavudine, lamivudine, efavirenz) was commenced 43 days after starting anti-tuberculosis treatment. The patients developed headache, paraparesis, T10 spinal sensory level and urinary and faecal incontinence 7 days after starting ART. Lumbar puncture performed showed protein 72 g/l, glucose 2.3 mmol/l, lymphocytes 86/ μ l. TB, fungal and bacterial cultures were negative. T1, T2 and T1 gadolinium enhanced sagittal magnetic resonance images performed (A, B, C) 20 days after symptom onset showed abnormal spinal cord signal from craniocervical junction to conus medullaris with cord expansion between C2–C6 vertebral bodies. Cerebrospinal fluid revealed signal abnormalities on both T1 and T2 weighted images. The cauda equina appeared thickened. Post gadolinium enhancement of arachnoid mater and epidural soft tissue surrounding the spinal cord as well as basal leptomeninges of the brain were present. Lung nodules were also evident. Diagnosis of paradoxical TB radiculomyelitis IRIS was made, with anti-tuberculosis treatment and ART being continued. She also received 58 days of prednisone started at an initial dose of 1.5 mg/kg/day 5 days prior to scan. There was no documented clinical improvement, presumably due to irreversible cord damage, but the patient was alive 3 months after starting ART and was then lost to follow-up. TB = tuberculosis; IRIS = immune reconstitution inflammatory syndrome; HIV = human immunodeficiency virus; ART = antiretroviral treatment.

Meningitis

TBM is the most frequent form of intracranial TB in HIV-1-infected²⁵ and non-infected²⁶ patients. Common neuroradiological findings in TBM include basal meningeal enhancement, hydrocephalus and infarction.^{20,26} In HIV-1-infected patients with TBM, these findings occur with the following frequencies: meningeal enhancement 23–33%,^{22,23,27} hydrocephalus 20–42%,^{23,27,28} obstructive hydrocephalus 5.5% and infarcts 39%.²²

In TBM, the presence of meningeal enhancement (33% vs. 82%) and obstructive hydrocephalus (5.5% vs. 64%) have previously been reported to be less frequent in profoundly immune-suppressed HIV-1-infected patients than in non-HIV-1-infected patients.²² This was ascribed to the attenuated immune response confirmed on histology. However, these findings are by no means consistent.^{23,27}

MRI features of TBM-IRIS have previously been

described in only three patients: meningeal enhancement developed on imaging subsequent to commencement of ART in one patient,¹² intra- and extra-axial lesions with peripheral contrast enhancement involving basal cisterns developed in another,¹³ and in the third, no meningeal enhancement was observed at the time of presentation.¹⁰

In our patient cohort, basal meningeal enhancement, which reflects inflammation, and the consequent hydrocephalus, were not seen with greater frequency in TBM-IRIS patients (1 of 9 patients) compared to HIV-1-infected patients with TBM not receiving ART.^{22,23,27,28} One explanation for this could be that more than half of this subgroup was receiving corticosteroids at the time of initial scanning. However, the use of corticosteroids has not been found to influence the degree of meningeal enhancement or reduce the development of hydrocephalus in non-HIV-1-infected adults with TBM.²⁹

Radiculomyelitis

TB radiculomyelitis is a relatively rare manifestation of CNS TB which may develop during the treatment of TBM.³⁰ The most consistent MRI finding is spinal cord hyperintensity on T2-weighted imaging.³¹ Loculation and obliteration of the subarachnoid space with post gadolinium intradural, leptomeningeal or cauda equina enhancement are also frequently noted.³⁰ Concurrent intracranial tuberculoma and TBM are common.³¹ Mycobacterial IRIS with spinal cord involvement has been described in two cases: one patient developed an expansive extra-axial thoracic lesion concurrent with a TBM-IRIS episode,¹³ while another patient with spinal mycobacterial IRIS (without confirmation of *M. tuberculosis*) presented with an enhancing intradural extramedullary spinal lesion.³²

Patient 16, with radiculomyelitis as a manifestation of TB-IRIS, initially presented with TBM before starting ART. At IRIS presentation, she developed rapid onset paraplegia; her MRI revealed leptomeningeal enhancement around the basal cisterns of the brain as well as marked enhancement of the spinal subarachnoid space.

Spondylitis

Classically, TB spondylitis affects two contiguous vertebral bodies (usually thoracolumbar),^{33,34} often with destruction of the intervertebral disc, and is associated with paravertebral abscess formation.³³ Involvement of a single vertebral body or posterior elements of the spine (especially when it is present without anterior element disease) occurs less frequently.³⁴ Patient 15 (previously reported by Dhasmana et al.³⁵) demonstrated osteitis of a single lumbar vertebra, notably with involvement of the vertebral body, pedicles and posterior elements. Although there was no significant paraspinal abscess, there was an epidural collection with resultant spinal stenosis.

Sequential radiological assessment of the development and resolution of lesions was not possible, as most patients did not have imaging performed either before or after initial assessment for neurological deterioration. In our setting, neuroradiological imaging, particularly MRI, is still relatively inaccessible compared to resource-rich settings. We did not use MRI in most of the patients with intracranial TB-IRIS. MRI may have detected more subtle cerebral lesions.

CONCLUSIONS

Although the largest series to date, our relatively small cohort of cases with various forms of neurological TB-IRIS and the absence of an appropriate control group precludes identification of a novel radiological marker of TB-IRIS. Our observations need to be confirmed in future prospective comparative studies.

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R É S U M É

CONTEXTE : Le syndrome inflammatoire paradoxal de reconstitution immunitaire chez les tuberculeux (TB-IRIS) constitue une complication importante chez les patients tuberculeux infectés par le virus de l'immunodéficience humaine (VIH) et placés sous traitement antirétroviral combiné (ART). Des manifestations neurologiques surviennent dans plus de 10% des cas TB-IRIS. A l'exception de quelques cas signalés, les caractéristiques radiologiques de la TB-IRIS neurologique n'ont pas été décrites.

OBJECTIF : Passer en revue les observations de 16 patients, dont 13 examinés par tomodensitométrie (CT) et trois par résonance magnétique.

RÉSULTATS : Les manifestations de l'IRIS ont comporté 4 méningites, 5 lésions situées dans l'espace intracrânien (SOL), probablement des tuberculomes, 5 cas de méningite avec SOL, une radiculomyélite et une spondylite. Chez les patients IRIS avec tuberculomes, nous avons

observé une prévalence élevée : 1) de lésions de faible densité dans les CT sans renforcement par produit de contraste (toutes les lésions) ; 2) des lésions multiples (chez 5 des 10 patients) et 3) de l'œdème périlésionnel dans 17 des 22 lésions. Chez les patients atteints de méningite, on n'a observé que peu fréquemment un épaississement méningé ($n = 2$) ou une hydrocéphalie ($n = 1$). **CONCLUSION :** Cette série est la première qui décrit de manière substantielle les caractéristiques radiologiques du TB-IRIS paradoxal avec atteinte méningée. Par comparaison avec les observations radiologiques publiées de tuberculome chez les patients infectés par le VIH mais non traités par ART, les tuberculomes IRIS s'accompagnent probablement d'une accentuation de la réaction inflammatoire. Celle-ci n'a toutefois pas été observée dans les méningites chez les patients IRIS.

R E S U M E N

MARCO DE REFERENCIA: El síndrome inflamatorio de reconstitución inmunitaria asociado con la tuberculosis (TB-IRIS) representa una complicación grave en los pacientes tuberculosos coinfectados por el virus de la inmunodeficiencia humana 1 (VIH-1), en quienes se inicia el tratamiento antirretrovírico (ART) combinado. En más del 10% de los casos se observan manifestaciones neurológicas. Aparte de algunos informes de casos, no se han descrito las características del estudio neurorradiográfico de la TB-IRIS.

OBJETIVO: Describir los hallazgos en 16 pacientes: 13 por tomografía computarizada y 3 por resonancia magnética.

RESULTADOS: Las manifestaciones del fueron meningitis ($n = 4$), lesiones intracraneales con efecto de masa (SOL; presunción de tuberculoma, $n = 5$), meningitis y

lesiones con SOL ($n = 5$), radiculomielitis ($n = 1$) y espondilitis ($n = 1$). En pacientes con IRIS y tuberculomas, se observó una alta frecuencia de: 1) lesiones de baja densidad o sin realce con el contraste en el escáner (en todas las lesiones); 2) lesiones múltiples (en 5 de 10 pacientes); y 3) edema perilesional (en 17 de 22 lesiones). En los pacientes con meningitis, rara vez se observó realce meníngeo ($n = 2$) o hidrocefalia ($n = 1$).

CONCLUSIÓN: Esta es la primera serie de casos importante donde se describen las características radiográficas de TB-IRIS. Comparados con los hallazgos publicados en pacientes infectados por el VIH-1 que no reciben ART, los tuberculomas IRIS indican una respuesta inflamatoria acentuada. Sin embargo, ese aspecto no se encontró en los pacientes con meningitis y IRIS.

**Warfarin Induced Skin Necrosis in HIV-1 Infected Patients with
Tuberculosis and Venous Thrombosis**

Running title: Warfarin induced skin necrosis

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ABSTRACT

Background: At the turn of the century, only 300 cases of warfarin induced skin necrosis (WISN) had been reported. WISN is a rare but potentially fatal complication of warfarin therapy. There are no published reports of WISN occurring in patients with HIV-1 infection or tuberculosis (TB).

Methods: We retrospectively reviewed 6 cases of WISN presenting from April 2005 – July 2008 at a referral hospital in Cape Town, South Africa.

Results: Six cases of WISN occurred in 973 patients receiving warfarin therapy for venous thrombosis (0.61%, 95% CI: 0.25-1.37%). All 6 cases occurred in HIV-1 infected women (median age: 30 years, range 27-42) with microbiologically confirmed TB and venous thrombosis. All patients were profoundly immune suppressed (median CD4 at TB diagnosis: 49 cells/mm³, interquartile range 23-170). Of the 3 patients receiving combination antiretroviral therapy, 2 had TB-IRIS. Median duration from initiation of antitubercular treatment to venous thrombosis was 37 days (range: 0-150). Median duration of parallel heparin and warfarin therapy was 2 days (range: 1-6). WISN manifested 6 days (range: 4-8) after initiation of warfarin therapy. The INR at WISN onset was supra-therapeutic, median: 6.2 (range: 3.8-6.6). Site of WISN included breasts, buttocks, and thighs. Four of six WISN sites were secondarily infected with drug-resistant nosocomial bacteria (MRSA, *Acinetobacter*, ESBL *E. coli* and *Klebsiella pn.*) 17 to 37 days after WISN onset. In four patients, median duration from WISN onset to death was 43 days (range: 25-45). One of the two surviving patients underwent bilateral mastectomies and extensive skin grafting at a specialist centre.

Conclusion: This is one of the largest case series of WISN. We report a novel clinical entity: WISN in HIV-1 infected patients with TB and venous thrombosis. The occurrence of 6 WISN cases in a 40 month period may be attributed to i) hypercoagulability, secondary to HIV-1 and TB, ii) short concurrent heparin and warfarin therapy, and iii) the high loading dose of warfarin. Active prevention and appropriate management of WISN will likely alleviate the dire morbidity and mortality of this unusual condition.

INTRODUCTION

Warfarin induced skin necrosis (WISN) is a rare complication of warfarin therapy, with an estimated prevalence of 0.01-0.1% in individuals receiving warfarin.^{1,2} WISN is associated with high morbidity, often necessitating aggressive surgical intervention, and may be fatal in the absence of early accurate diagnosis and treatment. Originally described in 1943, WISN was first associated with oral anticoagulants in 1954.³ As of 2000, only 300 case reports had been reported internationally.⁴ Most cases arise in patients receiving treatment for venous thromboembolism; 25% of WISN occurs in patients with cardiac indications for therapy (e.g. atrial fibrillation, valve replacement) or cerebral vascular insufficiency.² To date, published reports do not associate WISN with HIV-1 infection or tuberculosis. Here, we describe six cases of WISN with poor outcome, occurring in HIV-1 infected patients receiving treatment for tuberculosis.

METHODS

Setting

We retrospectively reviewed 6 cases of WISN at GF Jooste Hospital, Cape Town, South Africa from April 2005 through July 2008. GF Jooste Hospital is a 200-bed adult (≥ 15 years) public hospital, which receives referrals from primary care clinics, serving a catchment population of 1.3 million indigent people. We have previously described national guidelines for antitubercular treatment and antiretroviral therapy in South Africa.^{5,6}

Definitions

We defined venous thrombosis as either visualisation of a non-compressible thrombus with doppler ultrasound (popliteal or femoral venous thrombosis) or a venous filling defect with radio-contrast during computer tomography [CT] (inferior vena cava or superior sagittal sinus thrombosis). A radiologist performed sonography and interpreted CT findings. Warfarin induced skin necrosis (WISN) was defined as a characteristic drug eruption on the skin, which occurred shortly after starting warfarin therapy for a venous thrombosis, and which progressed to skin and subcutaneous tissue loss. We defined microbiologically confirmed tuberculosis as *Mycobacterium tuberculosis* (*M.tb*) cultured or acid-fast bacilli (AFB) seen in sputum or a lymph node aspirate. We defined TB-IRIS using the consensus clinical case definition of paradoxical TB-IRIS for resource-limited settings.⁷ We defined extended-spectrum beta lactamase (ESBL) producing bacteria as bacteria having clavulanate-inhibited transferable enzymes able to hydrolyse third and fourth generation cephalosporins as tested by disc diffusion (fishtail) method. We defined methicillin resistant *Staphylococcus aureus* (MRSA) as having an oxacillin minimum inhibitory concentration of $>4\text{mg/l}$.

Materials

We obtained clinical information from hospital notes, laboratory reports, and communication with attending physicians. The following data was reviewed: patient demographics, HIV-1 status, CD4 counts (nadir and post ART where available), tuberculosis episode (microbiological confirmation, drug susceptibility testing), site of venous thrombosis, anticoagulation therapy, site of WISN, international normalised ratio (INR) at WISN onset, antibiotic treatment, and outcome (e.g. death, surgical intervention, etc). All patients admitted to GF Jooste Hospital from 2005 through 2008 were managed using a standardised venous thrombosis protocol. Following venous thrombosis diagnosis, low molecular weight heparin (Enoxiparin 1 mg / kg twice daily by deep subcutaneous injection) was prescribed for a maximum of five days. Warfarin was started 2 days after heparin initiation to minimize the risk of warfarin-induced skin necrosis. If the patient had been on TB treatment for 10 days or longer, the loading dose of warfarin was adjusted from 5mg to 10mg.

The Research Ethics Committee of the University of Cape Town approved the study (REF:182/2009).

RESULTS

Baseline characteristics

Nine-hundred seventy three patients were diagnosed with venous thromboses and received warfarin therapy at GF Jooste Hospital over the 40-month study period. WISN occurred in six HIV-1 infected women receiving treatment for microbiologically confirmed tuberculosis (Table 1). The prevalence of WISN in our study population was 0.61%, 95% CI: 0.25-1.37% (6/973). The median age was 33 years (range: 27-42). The median CD4 count at TB diagnosis was 49 cells/mm³ (interquartile range: 23-170). Three patients received ART (regimens specified in Table 1). Two patients (Cases 2 and 3) were diagnosed with TB-IRIS. The median interval from initiation of antitubercular therapy to venous thrombosis was 37 days (range: 0-150). Venous thrombosis sites included popliteal and femoral veins, the inferior vena cava, and the superior sagittal sinus. Only Case 3 was an inpatient at the time of venous thrombosis (and received low molecular weight heparin prophylaxis); the remaining patients were admitted to hospital as a result of venous thrombosis. No patient had a personal or family history of previous venous thrombosis.

Clinical features at WISN and outcomes

The warfarin loading dose was 5mg or 10mg (Table 2). The median duration of parallel heparin and warfarin therapy was 2 days (range: 1-6). The median duration from initiation of warfarin therapy to WISN was 6 days (range: 4-8). The INR at WISN onset was supra-therapeutic, median: 5.6 (range: 3.8-6.6). Activated partial thromboplastin times (aPTT) were not measured. Site of WISN included breasts, buttocks, and thighs (Figure 1). Skin biopsy was performed in one patient (Figure 1). After WISN diagnosis, warfarin was stopped and low molecular weight heparin was used to manage anticoagulation. Wound cultures from

infected WISN sites produced the following drug-resistant nosocomial organisms: *E.coli* (ESBL), *Klebsiella pneumoniae* (ESBL), *Staph. aureus* (MRSA), *A.baumannii*, and *S.marcescens*. Antimicrobial sensitivities of each organism are listed in Table 2.

Four patients died, and no autopsies were performed. All 4 patients were profoundly immune-suppressed at TB diagnosis. The median duration from WISN onset to death was 43 days (range: 25-45). The two surviving patients' CD4 counts at TB diagnosis exceeded 200cells/mm³. Case 1 was referred to a specialist centre for aggressive surgical management (Figure 1 D-F). Case 6 recovered with appropriate wound care and prophylactic broad-spectrum intravenous antibiotics (3rd generation cephalosporin).

Table 1. Baseline characteristics and site of venous thrombosis in 6 HIV-1 infected patients with warfarin induced skin necrosis

Case	Age (years), sex	Previous TB	TB site, TB result	Duration: TB treatment to ART	ART regimen	TB-IRIS	CD4 at TB diagnosis (cells/mm ³)	CD4 at TB-IRIS (cells/mm ³)	Duration: ART to venous thrombosis	Duration: TB treatment to venous thrombosis	Venous thrombosis site
1	42, F	Yes	Pulmonary, drug sensitive <i>M.tb</i>	*	AZT/3TC/ EFV	–	396	-	28 months	0 days**	L popliteal vein
2	36, F	No	Cervical node, smear positive	4 weeks	D4T/3TC/ EFV	Yes	41	199	1 month	65 days	R SFV, R popliteal vein
3	30, F	No	Pulmonary, drug sensitive <i>M.tb</i>	2 weeks	D4T/3TC/ EFV	Yes	10	91	1 month	50 days	IVC, into L renal vein
4	28, F	Yes	Pulmonary, drug sensitive <i>M.tb</i>	–	–	–	56	–	–	0 days**	L popliteal and femoral veins
5	27, F	No	Pulmonary, smear positive	–	–	–	17	–	–	152 days	Superior sagittal sinus
6	35, F	No	Pulmonary, drug sensitive <i>M.tb</i>	–	–	–	208	–	–	24 days	L common femoral vein

Key:

F = female, NP = not performed, ART = combination antiretroviral treatment, AZT = zidovudine 300mg twice daily, D4T= stavudine 30mg twice daily, 3TC = lamivudine 150mg twice daily, EFV = efavirenz 600mg nocte, smear positive = acid fast bacilli seen with Ziehl Neelsen stain, drug sensitive *M.tb* = *Mycobacterium tuberculosis* cultured sensitive to rifampin and isoniazid, TB treatment = antitubercular treatment, TB-IRIS = tuberculosis associated immune reconstitution inflammatory syndrome, L = left, R = right, PE = pulmonary embolus, SFV = superficial femoral vein, IVC = inferior vena cava, * = ART preceded TB treatment by 28 months, 0 days** = tuberculosis and venous thrombosis diagnosed on the same day

Table 2. Clinical features at warfarin induced skin necrosis and outcomes

Case	Initial warfarin dosage (daily)	Duration of heparin + warfarin overlap	Duration from warfarin treatment to WISN onset	INR at WISN onset	Antibiotics at WISN onset	Site of WISN	Duration from WISN onset to wound infection	Wound culture from WISN site	Antimicrobial sensitivities	WISN to death
1	5mg	2 days	6 days	6.6	ART, HRZES, TMP-SMX, ceftriaxone	Breasts, L thigh	21 days	<i>S. aureus</i> (MRSA)	Clindamycin, erythromycin, vancomycin	Alive
								<i>A. baumannii</i>	Colistin	
2	10mg	3 days	4 days	6.2	ART, HRZE	R buttock	26 days	<i>Klebs pn</i> (ESBL),	Amikacin, ertapenem, imipenem, meropenem	44 days
								<i>E.coli</i>	Amikacin, cefotaxime, ceftriaxone, cefuroxime, ciprofloxacin, co-amoxyclav, gentamicin	
3	10mg	1 day	4 days	4.9	ART, HRZE, ampicillin, amikacin	Buttocks	17 days	<i>E. coli</i> (ESBL),	Amikacin, imipenem, meropenem, piperacillin-tazobactam	25 days
								<i>A. Baumannii</i>	Amikacin, colistin, tobramycin	
4	10mg	2 days	8 days	4.5	HRZE	L breast	37 days	<i>S.marcescens</i> ,	Imipenem, meropenem, piperacillin-tazobactam	42 days
								<i>Klebs pn</i> (ESBL)	Amikacin, imipenem, meropenem	
5	-	6 days	6 days	6.4	HRZE	L breast, L hip	-	Not requested		45 days
6	5mg	3 days	8 days	3.8	HRZE, Metronidazole TMP-SMX	L thigh	-	Not requested (wound not infected)		Alive

Key: WISN = warfarin induced skin necrosis; ART = combination antiretroviral treatment; HRZES = antitubercular treatment: H: isoniazid, R: rifampicin, Z: pyrazinamide, E: ethambutol, S: streptomycin; TMP-SMX: trimethoprim-sulfamethoxazole; INR = International Normalised Ratio; L = left, R = right; *S. aureus* = *Staphylococcus aureus*, MRSA = methicillin resistant *Staphylococcus aureus*, ESBL = extended spectrum beta-lactamase producing organism, *A. baumannii* = *Acinetobacter baumannii*, *Klebs pn* = *Klebsiella pneumoniae*, *E.coli* = *Escherichia coli*, *S.marcescens* = *Serratia marcescens*,

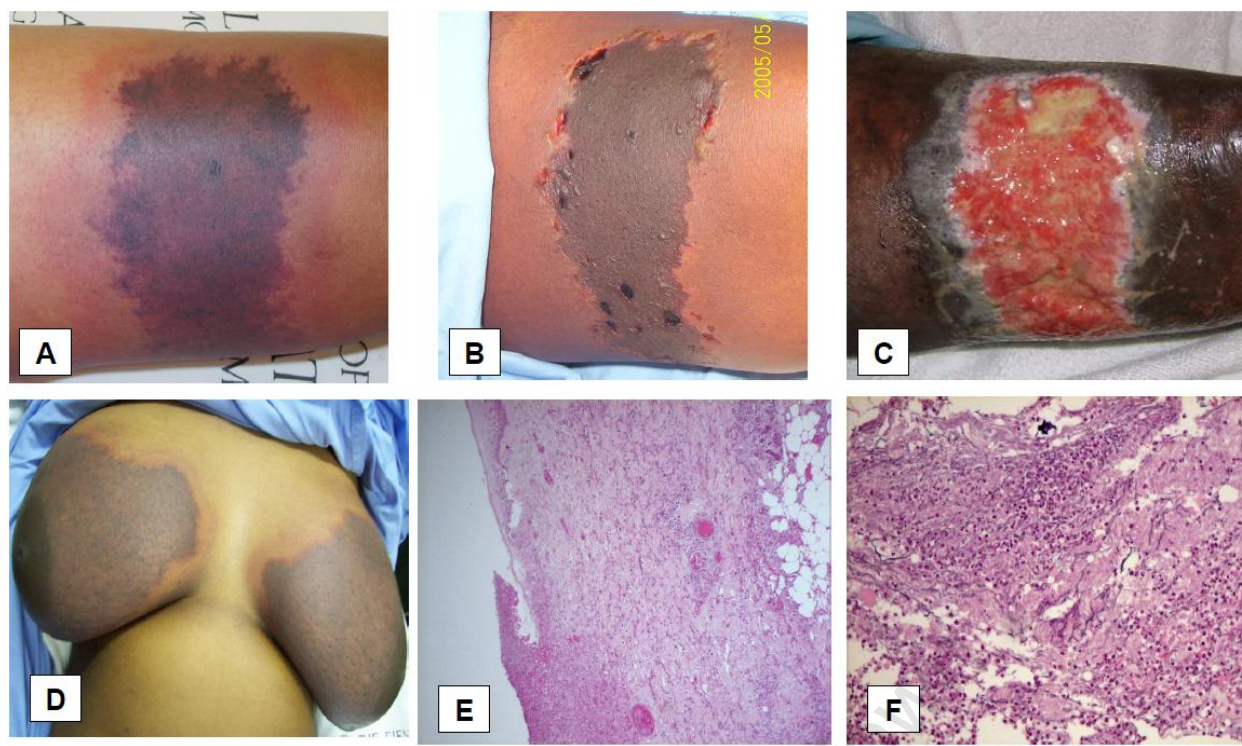


Figure 1: Left thigh of Case 6 showing typical macroscopic features of warfarin induced skin necrosis: A painful, well-localised purplish lesion with a poorly-demarcated erythematous flush⁹ developed 6 days after commencing warfarin therapy (A). Oedema formed in the dermis and subcutaneous tissues, which elevated and further demarcated the lesions from unaffected skin (B). Petechiae and hemorrhagic bullae were also observed; such bullae signified irreversible damage and ensuing full-thickness skin necrosis^{3,4,9}. Sloughing of the eschar revealed deep defects that extended into the subcutaneous tissue (C). **Warfarin induced skin necrosis occurring in a HIV-1 infected woman with tuberculosis and venous thrombosis (Case 1):** A 42 year-old HIV-infected woman was admitted to hospital in June 2008. She was compliant with antiretroviral treatment (ART), and her CD4 count was 396 cells/mm³. Drug-sensitive *Mycobacterium tuberculosis* was cultured from her sputa. Ultrasound confirmed a left popliteal thrombosis. Six days after starting warfarin therapy, she developed skin lesions on her breasts and left thigh, consistent with warfarin induced skin necrosis (WISN) (D). Sixteen days after WISN onset, a punch biopsy of her left thigh was performed. Histology showed full thickness epidermal necrosis with numerous thrombosed vessels in the superficial dermis (E, arrowed), consistent with WISN. She received antibiotics for wound infection according to microbial sensitivities. 44 days after WISN onset, plastic surgeons performed a bilateral mastectomy and an extensive tissue excision from her left thigh. Split thickness skin grafts were used to cover the defects. Microscopy of tissue excised from her left thigh showed extensive necrosis of subcutaneous fat, numerous foreign body-type giant cells and focal suppuration (F, arrowed). There were fresh thrombi in vessels, and some vessels showed recanalisation. No organisms were seen on Brown and Brenn (modified Gram stain) or Ziehl Neelsen stains. 10 months after WISN, she has an impaired gait due to a contracture of her left thigh.

DISCUSSION

This is one of the largest case series of warfarin induced skin necrosis (WISN). We report a novel clinical entity: WISN occurring in HIV-1 infected patients with tuberculosis and venous thrombosis.

All 6 patients were chronically ill women of reproductive-age with venous thromboses. WISN typically occurs in obese, perimenopausal women who are receiving anticoagulant therapy for a deep vein thrombosis or pulmonary embolism.² Women are affected more frequently than men (4:1)² – the reason for this predilection is unclear. In women, the breast is most commonly affected, followed by the buttocks and thighs;⁸ our patients were similarly affected. It is postulated that local tissue factors contribute to the development of WISN at these sites; such factors include trauma and variation in local temperature and perfusion.^{9,10}

About 90% of affected patients develop symptoms between the third and sixth day of warfarin therapy,^{2,4,11} which is similar to our experience. The clinical presentation of WISN is characteristic (Figure 1 A-C); all our patients demonstrated these clinical features.

Widespread disease may result in deep tissue necrosis, secondary infection, and multi-organ failure.²

Mortality within 3 months of WISN onset is substantial (15%), even with appropriate treatment.² Four of our 6 patients died within 45 days of WISN onset. All deaths were probably due to sepsis syndrome complicating wound infection. All wound cultures were taken from infected wounds. These wound cultures were not surveillance cultures. Prior

rifampicin, trimethoprim-sulfamethoxazole and cephalosporin use in our patients may have favoured the selection of highly antibiotic resistant organisms. In South Africa, more than 50% of *Staph. aureus* isolates from public hospitals are resistant to either rifampicin and/or trimethoprim sulfamethoxazole.¹² Failure of effective infection control measures, lack of appropriate antimicrobial chemotherapy,⁶ delayed referral to a specialist centre for surgical debridement, and profound immune-suppression at TB diagnosis probably contributed to the 4 deaths. Comparatively, the superior immune function at TB diagnosis of Cases 1 and 6 may account for their survival. Pulmonary emboli may have also complicated warfarin cessation and contributed to death. We were unable to determine the exact cause of death; relatives refused permission to perform autopsies. It is important to note that despite prompt referral to a specialist centre, Case 1 has considerable morbidity. This morbidity includes bilateral mastectomies, an impaired gait due to a contracture of her left thigh, and the associated psychosocial stigma.

Histology of WISN classically shows full thickness epidermal necrosis with thrombosed vessels in the dermis. While the underlying pathophysiological mechanisms remain unclear, it is postulated that WISN results from an imbalance between intrinsic pro- and anticoagulant factors during the first few days of warfarin therapy.^{2,11} Warfarin is a vitamin K antagonist and reduces serum levels of vitamin K-dependant factors, which include factors II, VII, IX, X, protein C, and protein S. Serum levels of factor VII (procoagulant factor), and protein C and protein S (anticoagulant factors) decline more rapidly than serum levels of factors II, IX, and X (procoagulant factors) on warfarin therapy.^{2,11} This results in an initial hypercoagulable state, which, especially in the presence of additional risk factors such as protein C and/or S deficiency, may predispose to WISN.⁴ The INR is factor VII dependent, thus patients will have a raised INR, yet still have a hypercoagulable state because of a

relative protein C deficiency.¹³ Screening for these conditions prior to warfarin commencement, however, is not recommended, as they lack the necessary sensitivity and specificity to accurately predict the risk of developing WISN.^{2,4} Due to the retrospective nature of our study, serum levels of protein C, protein S, and antithrombin III were not performed. The lack of genetic testing and coagulation work-up is a limitation of our study.

The prevalence of WISN in our study population is 0.61% (6/973), which is six times higher than reported in HIV un-infected patients.^{1,2} The occurrence of 6 WISN cases in a 40-month period at one centre is unusual. This may be a result of the short duration of parallel heparin and warfarin therapy (median duration 2 days), observed in our patients. Parallel heparin and warfarin therapy is postulated to prevent the development of WISN, and should be continued until the vitamin K-dependent clotting factors have been consumed (72-96 hours).^{2,4,8,11} In our patients, premature cessation of heparin during the initial hypercoagulable period of warfarin therapy may have exacerbated an underlying hypercoagulable disorder (such as a protein C or S deficiency) and culminated in WISN. In our setting, we routinely prescribe a loading dose of 5 or 10mg warfarin in tuberculosis patients with venous thromboses as rifampin induces the rate of warfarin clearance by cytochrome p450 (CYP) 2C9.¹⁴ This dose of warfarin with a short window of parallel heparin and warfarin therapy may have contributed to the high prevalence of WISN (0.61%).

HIV infection is a widely acknowledged risk factor for venous thromboembolism (VTE).¹⁵⁻¹⁷ Some reports cite a tenfold increase in incidence of DVT in HIV/AIDS as compared to the general population.¹⁵ The following independent risk factors have been identified for VTE in HIV-positive patients: low CD4 count, high viral load, advanced stage of

immunocompromise, opportunistic infections, AIDS-related neoplasms, HIV-associated autoimmune disorders (eg. AIHA), hospitalisation in the past 3 months, and central venous catheter use in the past 3 months.¹⁶⁻¹⁸ Exposure to HAART has not been associated with VTE.^{16,17} HIV-positive patients are also more likely to demonstrate multiple acquired and persistent thrombophilic abnormalities; the frequency of these abnormalities increases with progression to AIDS, and the presence of such abnormalities may contribute to the high prevalence of venous and arterial thrombosis in patients with HIV infection.¹⁹ These abnormalities include antiphospholipid antibodies, lupus anticoagulant, anticardiolipin antibodies, increased von Willebrand factor, increased d-dimers, and deficiencies of protein C, protein S, antithrombin, and heparin cofactor II.²⁰ The acquired protein S and protein C deficiencies seen in acutely ill patients may be reversible following treatment for OIs and/or ART.¹⁸

M.tb infection may present clinically as DVT; 2 of our patients (Cases 1 and 4) were diagnosed with TB and DVT simultaneously. DVT usually occurs shortly after initiating antitubercular therapy (about 2 weeks).²¹ Rifampin-based regimens have a fivefold increased risk of DVT (relative risk = 5), thus DVT prevention is recommended in patients on rifampicin.²¹ DVT is associated with advanced HIV infection and PTB. The following thrombogenic factors probably contribute to this association: acquired protein C and protein S deficiencies, elevated plasma fibrinogen, impaired fibrinolysis, depressed ATIII, reactive thrombocytosis, increased platelet aggregation, and antiphospholipid antibodies.²² These parameters may improve with antitubercular treatment.²²

It is not known whether IRIS predisposes to venous thrombosis. A single case is reported of IRIS manifesting as disseminated tuberculosis, myelopathy, encephalopathy, and deep vein thrombosis;²³ with appropriate treatment, IRIS resolved and no adverse drug effects occurred. We report the first two cases of TB-IRIS and WISN occurring simultaneously. The 2 patients diagnosed with TB-IRIS were profoundly immune-suppressed, had a short duration from starting antitubercular treatment to initiation of ART, and presented with recurrence of tuberculosis symptoms soon after initiating ART.⁷

Active prevention and appropriate management of venous thromboses will likely alleviate the dire morbidity and mortality associated with WISN. Prophylactic heparinisation of acutely ill hospital patients with HIV-1 infection and/or tuberculosis will reduce the incidence of venous thrombosis. In patients with venous thrombosis, parallel heparin therapy for at least the first 4 days of warfarinisation^{2,4,8,11} may limit the occurrence of WISN. WISN should be considered in all newly-warfarinised patients with new skin lesions. Effective infection control measures and expedited referral to specialist centres for surgical review may reduce mortality.

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Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings

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Abstract

The immune reconstitution inflammatory syndrome (IRIS) has emerged as an important early complication of antiretroviral therapy (ART) in resource-limited settings, especially in patients with tuberculosis. However, there are no consensus case definitions for IRIS or tuberculosis-associated IRIS. Moreover, previously proposed case definitions are not readily applicable in settings where laboratory resources are limited. As a result, existing studies on tuberculosis-associated IRIS have used a variety of non-standardised general case definitions. To rectify this problem, around 100 researchers, including microbiologists, immunologists, clinicians, epidemiologists, clinical trialists, and public-health specialists from 16 countries met in Kampala, Uganda, in November, 2006. At this meeting, consensus case definitions for paradoxical tuberculosis-associated IRIS, ART-associated tuberculosis, and unmasking tuberculosis-associated IRIS were derived, which can be used in high-income and resource-limited settings. It is envisaged that these definitions could be used by clinicians and researchers in a variety of settings to promote standardisation and comparability of data.

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Conflicts of interest We declare that we have no conflicts of interest.

Introduction

The immune reconstitution inflammatory syndrome (IRIS; also known as immune reconstitution disease, immune reconstitution syndrome, or immune restoration disease) is a widely recognised phenomenon that can complicate antiretroviral therapy (ART).^{1,2} The condition results from rapid restoration of pathogen-specific immune responses to opportunistic infections, causing either the deterioration of a treated infection or the new presentation of a previously subclinical infection. IRIS typically occurs during the initial months of ART and is associated with a wide spectrum of pathogens, most commonly mycobacteria, herpes-viruses, and deep fungal infections such as cryptococcal meningitis.¹⁻³

In recent years, access to ART has increased rapidly in resource-limited settings, reaching over 2 million people by December, 2006, with an estimated 1 340 000 of these individuals living in sub-Saharan Africa.⁴ Since the burden of HIV/tuberculosis co-infection is very high in many low-income and middle-income countries,⁵ many of the patients who enter ART programmes in these settings have a current diagnosis of tuberculosis, or later develop tuberculosis following initiation of ART. For example, one South African study reported that 238 (25%) of 944 patients attending a community-based ART programme were receiving tuberculosis treatment at ART initiation and in the first year of ART the incidence of tuberculosis was 13.4 cases per 100 person-years (95% CI 10.4–16.9).⁶ Up to one-third of patients with HIV/tuberculosis co-infection who begin ART in such settings could be at risk of developing tuberculosis-associated IRIS (also known as TB-IRIS),³ and this condition is emerging as an important clinical challenge in resource-limited settings.⁷⁻¹⁰

Since there is no diagnostic test for IRIS, confirmation of the disease relies heavily upon case definitions incorporating clinical and laboratory data. However, clinical management and research on IRIS are hindered by the lack of consensus case definitions and definitions that are specific to particular opportunistic infections. To address this shortcoming, an international meeting of researchers working in this field was convened in Kampala, Uganda, in November, 2006, and the International Network for the Study of HIV-associated IRIS (INSHI) was formed. The specific aim of the meeting was to develop consensus case definitions for tuberculosis-associated IRIS that are appropriate for low-income settings where laboratory capacity is often limited, and that can be used by researchers working in different settings to permit comparability of results. We present these consensus case definitions in this paper.

Participants and consensus methods

The need for a public-health definition for tuberculosis-associated IRIS was first proposed at the WHO consultation on tuberculosis and HIV research priorities in resource-limited settings in February, 2005.¹¹ The organisers of the meeting in Kampala contacted individuals involved in research related to tuberculosis-associated IRIS, particularly those working in resource-limited settings or collaborating with researchers in these settings. Contacting these individuals was dependent on whether they had published or presented data about tuberculosis-associated IRIS at international conferences, whether they were involved in ongoing research projects about the disease, or whether they had clinical experience of the disease. 97 researchers from 16 countries on six continents attended the meeting. Among the delegates were microbiologists, immunologists, clinicians, epidemiologists, clinical trialists, public-health specialists, and representatives from WHO.

At the meeting a subgroup was assembled to develop the case definitions. Two participants presented published IRIS case definitions (panel 1)^{2,7,12-14} as well as eight different tuberculosis-associated IRIS case definitions currently being used by researchers in ongoing

cohort and intervention studies. The common features among these case definitions were highlighted, and their practical use in resource-limited settings was discussed. Tuberculosis-associated IRIS case definitions were agreed and taken back to a plenary session for further discussion and consensus building. Thereafter,

Panel 1

Existing case definitions for IRIS and tuberculosis-associated IRIS that have been most widely used

General IRIS case definition 1 (French et al, 2004)²

Diagnosis requires two major criteria (A+B) or major criterion (A) plus two minor criteria to be fulfilled:

Major criteria

(A) Atypical presentation of opportunistic infections or tumours in patients responding to ART

- Localised disease
- Exaggerated inflammatory reaction
- Atypical inflammatory response in affected tissues
- Progressive organ dysfunction or enlargement of pre-existing lesions after definite clinical improvement with pathogen-specific therapy before the initiation of ART and exclusion of treatment toxicity and new alternative diagnoses

(B) Decrease in plasma HIV RNA concentration by more than 1 log₁₀ copies per mL

Minor criteria

- Increase in blood CD4 T-cell count after starting ART
- Increase in an immune response specific to the relevant pathogen—eg, delayed-type hypersensitivity skin test response to mycobacterial antigens
- Spontaneous resolution of disease without specific antimicrobial therapy or tumour chemotherapy with continuation of ART

General IRIS case definition 2 (Shelburne et al, 2006)¹³

Criteria for IRIS diagnosis include:

- HIV-infected patient
- Receiving effective ART as evidenced by a decrease in HIV-1 RNA concentration from baseline or an increase in CD4+ T cells from baseline (may lag behind HIV-1 RNA decrease)
- Clinical symptoms consistent with inflammatory process
- Clinical course not consistent with expected course of previously diagnosed opportunistic infection, expected course of newly diagnosed opportunistic infection, or drug toxicity

Case definition specific for tuberculosis-associated IRIS (Colebunders et al, 2006)⁷

For patients receiving treatment for tuberculosis and starting ART:

Suspected tuberculosis-associated IRIS case

Cases must meet the following three criteria:

- An initial clinical response to tuberculosis treatment, based on a combination of some of the following factors: cessation of fever, relief of pulmonary symptoms, decrease in lymph node size, termination of signs of meningeal irritation (depending on presenting symptoms)
- New persistent fevers without another identifiable cause and/or one or more of the following: worsening or emergence of dyspnoea, stridor, an increase in lymph node size, development of abscesses, development of abdominal pain with ultrasound evidence of abdominal adenopathies, unexplained CNS symptoms
- Adequate adherence to ART and tuberculosis treatment

Confirmed tuberculosis-associated IRIS case

Cases must meet the following three criteria:

- Radiological examinations showing worsening or emergence of intrathoracic lymphadenopathy, pulmonary infiltrates, pleural effusions, abdominal lymph nodes, hepatosplenomegaly
- A good virological response and/or increase in CD4+ lymphocyte count, and/or conversion of tuberculin skin test from negative to positive, and/or adequate adherence to ART and tuberculosis treatment
- A clear exclusion of other conditions that could explain the clinical manifestations of the patient, such as tuberculosis treatment failure or other concomitant infections, tumours, or allergic reactions

ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome.

a writing committee of 17 members was appointed to finalise the consensus case definitions.

Changes to existing case definitions

General case definitions for IRIS have previously been published (panel 1).^{2,12-14} These case definitions include the following criteria: confirmed HIV diagnosis, temporal association with initiation of ART, demonstration of response to ART (ie, plasma viral load reduction, blood CD4 cell count increase, or another marker of immune recovery such as conversion of tuberculin skin test from negative to positive for mycobacterial IRIS), clinical deterioration with an inflammatory process, and exclusion of other causes that could explain deterioration (such as antimicrobial drug resistance, drug hypersensitivity reaction, or another opportunistic infection). However, since manifestations of IRIS are infection-specific, it has been recognised that particular definitions applicable to individual diseases such as tuberculosis would be useful.⁷ The case definitions presented in this manuscript focus specifically on the clinical manifestations of tuberculosis-associated IRIS.

Case definitions should be readily applicable in resource-limited settings where the vast majority of patients requiring ART live and yet where facilities for diagnosis and management of the complications of ART are least well developed. In this respect, the requirement within existing definitions for documentation of changes in CD4 cell count and plasma viral load is not achievable in these settings. Viral load testing has limited availability and is very costly. In the South African public sector a viral load test costs US\$39, more than the cost of 1 month's supply of first-line ART. Even where CD4 and viral load testing are available (such as in South Africa), use of these tests under programmatic conditions is usually permitted for monitoring of ART at 6-monthly intervals only and not for individual patient diagnostic work-up.

We believe that omission of these laboratory parameters would not substantially compromise case definitions for tuberculosis-associated IRIS. First, within the initial months of ART—when most cases of tuberculosis-associated IRIS arise—most ART-naïve patients adhering to treatment have substantial viral load reductions;^{15–17} thus, inclusion of viral load changes in definitions is largely redundant in the context of a patient who adheres to therapy. Second, tuberculosis-associated IRIS frequently develops shortly after initiation of ART and before any measurable increase in peripheral blood CD4 cell count. In a series of 51 patients presenting with non-tuberculous mycobacterial IRIS, six (12%) of 51 IRIS events occurred without a substantial increase in CD4 cell count (four patients had a CD4 increase from baseline to time of IRIS diagnosis of less than 25 cells per μL and in two patients the CD4 cell count had actually fallen at the time of presentation).¹⁸ The number of CD4 T cells measured in peripheral blood does not necessarily reflect function nor how many cells are actually present at the site of an opportunistic infection. Moreover, it is very likely that CD4 T cells are not the only cellular mediators of IRIS.^{19,20} For these reasons we, like others,¹² propose that a rise in peripheral blood CD4 cell count should not be a necessary marker for the diagnosis of tuberculosis-associated IRIS.

A further important modification to existing definitions is the inclusion of a timeframe of the first 3 months of ART. Such a timeframe is not present in the widely used case definitions to date (panel 1). Onset of the clinical manifestations of tuberculosis-associated IRIS should occur within this timeframe for a diagnosis of tuberculosis-associated IRIS to be made, since this represents the period when rapid immune recovery usually occurs.^{3,21}

Categories of tuberculosis-associated IRIS

Tuberculosis-associated IRIS can present as one of two main syndromes: (1) a paradoxical reaction after the start of ART in patients receiving tuberculosis treatment (here termed paradoxical tuberculosis-associated IRIS), or (2) a new presentation of tuberculosis that is “unmasked” in the weeks following initiation of ART with an exaggerated inflammatory clinical presentation or complicated by a paradoxical reaction (here termed unmasking tuberculosis-associated IRIS).

Paradoxical tuberculosis-associated IRIS

In paradoxical tuberculosis-associated IRIS, patients have been diagnosed with active tuberculosis before initiation of ART, and have typically been responding to antituberculosis treatment. Following initiation of ART, IRIS presents as the development of recurrent, new, or worsening symptoms or signs of tuberculosis, such as fever, return of cough, or lymph node enlargement, or recurrent, new, or deteriorating radiological manifestations (figure 1). These symptoms typically occur within the first few weeks and up to 3 months after ART is initiated, restarted, or changed because of treatment failure.

Reports of the frequency of paradoxical tuberculosis-associated IRIS using a variety of existing case definitions range from 8% to 43% (table).^{8–10,21–28} Paradoxical tuberculosis-associated IRIS has been linked with large expansions of purified protein derivative-specific T cells in peripheral blood and increased pro-inflammatory cytokine levels.²⁹ Risk factors for the disease are shown in table 1 and include more advanced HIV disease with lower CD4 cell count, disseminated and extrapulmonary tuberculosis, a shorter delay between the start of tuberculosis treatment and initiation of ART, and a more vigorous immunological and virological response to ART. Most cases of paradoxical tuberculosis-associated IRIS are self-limiting. The median duration of symptoms reported in the literature is 2 months,^{26,28} but this ranges from mild cases where symptoms resolve after a few days to isolated prolonged cases that have still been symptomatic after more than a year (figure 1).²⁸ Mortality from tuberculosis-associated IRIS has been reported infrequently in the literature,^{3,9,10,26} but morbidity and the need for hospital

admission and therapeutic procedures can be substantial.²⁶ Rates of morbidity and mortality attributable to paradoxical tuberculosis-associated IRIS may be higher in resource-limited settings where diagnostic and treatment options are restricted. Neurological tuberculosis-associated IRIS in particular can be associated with poor outcome.

Tuberculosis paradoxical reactions, such as enlargement of lymph nodes or cerebral tuberculomas, can also occur in HIV-uninfected individuals and HIV-infected individuals who are receiving appropriate tuberculosis treatment but who are not receiving ART;^{30–32} however, the frequency of paradoxical reactions is much lower in these groups compared with patients receiving ART.^{22,23} In one study, paradoxical reactions following tuberculosis treatment occurred in one (2%) of 55 HIV-seronegative patients, two (7%) of 28 HIV-infected patients not on ART, and 12 (36%) of 33 HIV-infected patients on tuberculosis treatment and ART.²² The timing of the paradoxical reaction in the latter group was more closely related to the initiation of ART than it was to the initiation

Panel 2

Case definition for paradoxical tuberculosis-associated IRIS

There are three components to this case definition:

(A) Antecedent requirements

Both of the two following requirements must be met:

- Diagnosis of tuberculosis: the tuberculosis diagnosis was made before starting ART and this should fulfil WHO criteria for diagnosis of smear-positive pulmonary tuberculosis, smear-negative pulmonary tuberculosis, or extrapulmonary tuberculosis⁴⁴
- Initial response to tuberculosis treatment: the patient's condition should have stabilised or improved on appropriate tuberculosis treatment before ART initiation—eg, cessation of night sweats, fevers, cough, weight loss. (Note: this does not apply to patients starting ART within 2 weeks of starting tuberculosis treatment since insufficient time may have elapsed for a clinical response to be reported)

(B) Clinical criteria

The onset of tuberculosis-associated IRIS manifestations should be within 3 months of ART initiation, reinstitution, or regimen change because of treatment failure.

Of the following, at least one major criterion or two minor clinical criteria are required:

Major criteria

- New or enlarging lymph nodes, cold abscesses, or other focal tissue involvement—eg, tuberculous arthritis
- New or worsening radiological features of tuberculosis (found by chest radiography, abdominal ultrasonography, CT, or MRI)
- New or worsening CNS tuberculosis (meningitis or focal neurological deficit—eg, caused by tuberculoma)
- New or worsening serositis (pleural effusion, ascites, or pericardial effusion)

Minor criteria

- New or worsening constitutional symptoms such as fever, night sweats, or weight loss

- New or worsening respiratory symptoms such as cough, dyspnoea, or stridor
- New or worsening abdominal pain accompanied by peritonitis, hepatomegaly, splenomegaly, or abdominal adenopathy

(C) Alternative explanations for clinical deterioration must be excluded if possible*

- Failure of tuberculosis treatment because of tuberculosis drug resistance
- Poor adherence to tuberculosis treatment
- Another opportunistic infection or neoplasm (it is particularly important to exclude an alternative diagnosis in patients with smear-negative pulmonary tuberculosis and extrapulmonary tuberculosis where the initial tuberculosis diagnosis has not been microbiologically confirmed)
- Drug toxicity or reaction

ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome. *It might be difficult or impossible in resource-poor settings to confirm tuberculosis drug resistance and to exclude certain other infections or neoplasia. Cases where alternative diagnoses cannot be fully excluded because of limited diagnostic capacity should be regarded as “probable paradoxical tuberculosis-associated IRIS”. In these probable cases, should resolution of clinical or radiological findings of the suspected IRIS episode occur without a change in tuberculosis treatment or ART having been made, they could then be reclassified as “paradoxical tuberculosis-associated IRIS” cases.

of tuberculosis treatment. Thus, the greatly increased frequency of paradoxical reactions in patients receiving ART suggests that ART-related immunological changes have an important role in their aetiology. Additionally, our clinical experience is that paradoxical tuberculosis-associated IRIS is more severe and more frequently a multisystemic condition in ART patients than paradoxical reactions in patients not receiving ART.

ART-associated tuberculosis and unmasking tuberculosis-associated IRIS

Compared with paradoxical tuberculosis-associated IRIS, there is much less clarity surrounding the second major category of tuberculosis-associated-IRIS. High rates of tuberculosis have been diagnosed during ART, especially in the initial months of treatment in ART programmes in resource-limited settings.^{6,33–36} The mechanisms underlying the presentation of tuberculosis after the initiation of ART are likely to be heterogeneous.³⁷ Since ART-induced immune recovery is a time-dependent process and some patients initially fail to show an increased circulating CD4 T-cell count,^{38,39} a proportion of cases might present as a result of persisting immunodeficiency. Diagnoses of active tuberculosis before ART initiation might be missed because of the inherent insensitivity of tuberculosis diagnostics in patients with advanced immunodeficiency and only confirmed later during ART. Other patients might have active subclinical disease at the time of ART initiation and presentation of symptomatic disease might result from ART-induced restoration of an immune response against *Mycobacterium tuberculosis* antigens that causes inflammation. Some patients with a missed tuberculosis diagnosis or active subclinical tuberculosis at the time of ART initiation may later present with exuberant inflammatory clinical features that are consistent with a diagnosis of unmasking tuberculosis-associated IRIS (figure 2).

Paradoxical reactions in patients started on tuberculosis treatment while receiving ART have also been described,^{26,40} and one study reported that paradoxical reactions are more frequent in patients who are diagnosed with tuberculosis in the first 3 months of ART than in patients who start ART after tuberculosis treatment (eight [62%] of 13 patients vs nine [30%] of 30

patients, respectively, $p=0.05$).⁴⁰ This finding suggests that ART-related immunological changes have a role in the development of paradoxical reactions in patients who present with tuberculosis while receiving ART and that these reactions are a form of tuberculosis-associated IRIS.

Only a few cases of unmasking tuberculosis-associated IRIS have been described in the literature to date.⁴⁰⁻⁴³ In the absence of a diagnostic test, it is currently difficult to differentiate the varied mechanisms underlying most cases of tuberculosis that present during early ART, especially in resource-limited settings where rates of infection are high. We therefore propose that, as elsewhere,³⁷ the term ART-associated tuberculosis is used

Panel 3

Case definition for ART-associated tuberculosis and provisional case definition for unmasking tuberculosis-associated IRIS

ART-associated tuberculosis

We propose that ART-associated tuberculosis (all cases of tuberculosis that are diagnosed during ART) should be defined as follows:

- Patient is not receiving treatment for tuberculosis when ART is initiated
- Active tuberculosis is diagnosed after initiation of ART
- The diagnosis of tuberculosis should fulfil WHO criteria for smear-positive pulmonary tuberculosis, smear-negative pulmonary tuberculosis, or extrapulmonary tuberculosis⁴⁴

Unmasking tuberculosis-associated IRIS (provisional)

We propose that the following could suggest a diagnosis of unmasking tuberculosis-associated IRIS:

- Patient is not receiving treatment for tuberculosis when ART is initiated and then presents with active tuberculosis within 3 months of starting ART

AND one of the following criteria must be met:

- Heightened intensity of clinical manifestations, particularly if there is evidence of a marked inflammatory component to the presentation. Examples include tuberculosis lymphadenitis or tuberculosis abscesses with prominent acute inflammatory features, presentation with pulmonary tuberculosis that is complicated by respiratory failure due to adult respiratory distress syndrome, and those who present with a marked systemic inflammatory syndrome related to tuberculosis. See example in figure 2
- Once established on tuberculosis treatment, a clinical course that is complicated by a paradoxical reaction

ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome. Researchers in the field are encouraged not to regard all patients with ART-associated tuberculosis as having tuberculosis-associated IRIS, but only those that fit this provisional unmasking tuberculosis-associated-IRIS case definition. We suggest that the clinical manifestations of all patients developing ART-associated tuberculosis should be well characterised and reported in studies, which will assist with refinement of this case definition in the future. Studies of the immunological processes underlying the presentation of these cases are also likely to assist with refining this case definition.

to refer to all patients who present with active tuberculosis while receiving ART (figure 3). We also suggest a provisional case definition for unmasking tuberculosis-associated IRIS and clinical scenarios where the diagnosis could be considered.

Further research into the clinical characteristics and immunological mechanisms underlying cases of ART-associated tuberculosis will permit a more refined case definition for unmasking tuberculosis-associated IRIS in the future. However, in view of the heterogeneity in the natural history and clinical manifestations of tuberculosis it is unlikely that a clinical case definition that robustly separates patients with unmasking tuberculosis-associated IRIS from others with ART-associated tuberculosis will be derived.³⁷

Case definitions

With the rationale described above, we have developed case definitions for “paradoxical tuberculosis-associated IRIS” (panel 2), “ART-associated tuberculosis” (panel 3), and “unmasking tuberculosis-associated-IRIS” (panel 3). The case definitions are presented schematically in figure 3. These case definitions have been designed for use in resource-limited settings and are consensus case definitions that need validation in clinical practice.

Search strategy and selection criteria

Data for this Personal View were obtained by searching Medline for articles published from 1990 to 2008. Search terms included “immune reconstitution”, “immune restoration”, “immune recovery”, “IRIS”, “antiretroviral”, “tuberculosis”, and “paradoxical reaction”. Only English language papers were reviewed. Additionally, unpublished data and tuberculosis-associated IRIS case definitions presented by researchers at the INSHI meeting were used.

Conclusions

The use of standardised case definitions in different populations will help to provide greater insight into the incidence, clinical manifestations, risk factors, and impact of tuberculosis-associated IRIS, ultimately leading to better prevention and management strategies for this condition. Further clinical and immunological research on patients with ART-associated tuberculosis is needed to better differentiate the subset of cases that have unmasking tuberculosis-associated IRIS and to further refine this case definition. It is hoped that open research networks such as INSHI will provide opportunities for researchers to engage in collaborative research into tuberculosis-associated IRIS using these case definitions.

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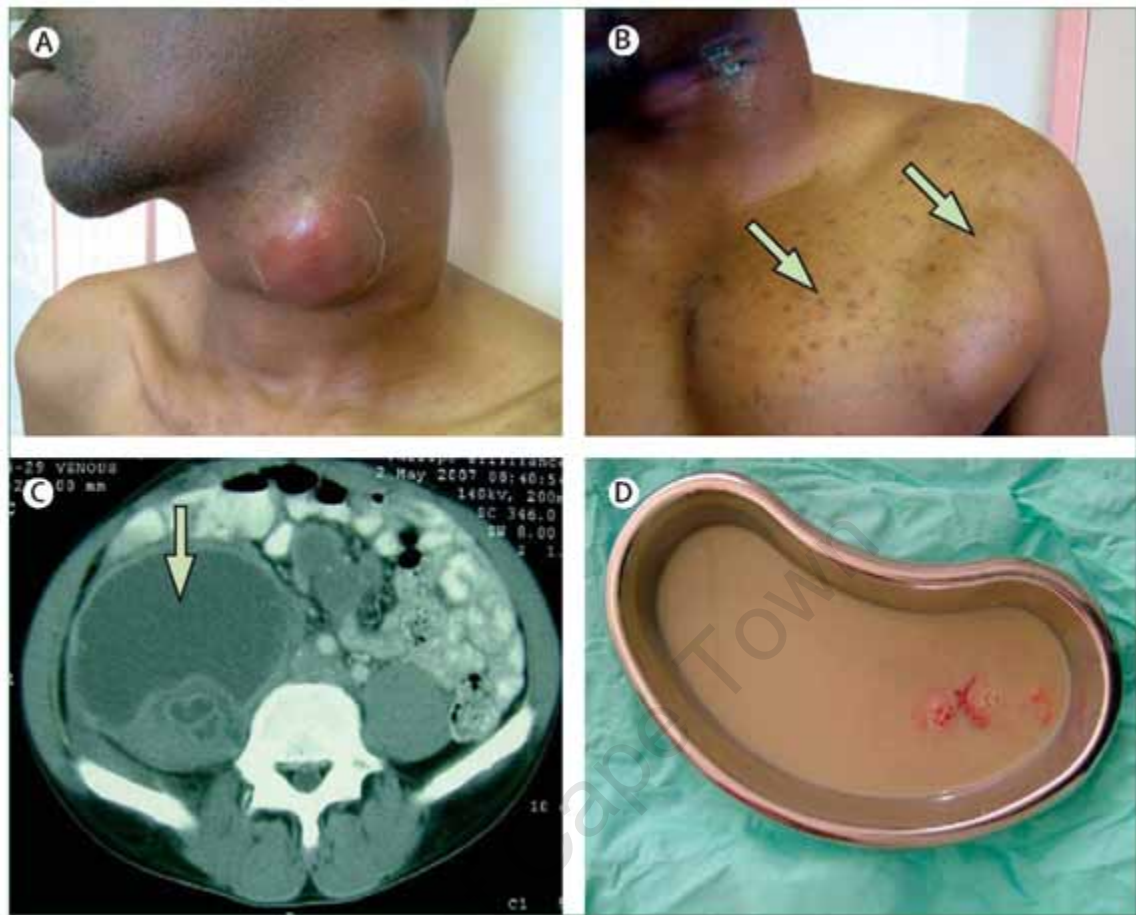


Figure 1. Illustrative case of paradoxical tuberculosis-associated IRIS

A 36-year-old HIV-infected man was diagnosed with culture-positive pulmonary tuberculosis (sensitive to rifampicin and isoniazid) without evidence of extrapulmonary involvement. His CD4 count was 39 cells per μL and HIV-1 viral load 1 300 000 copies per mL. He commenced antiretroviral therapy (ART; stavudine, lamivudine, and efavirenz) 7 weeks after initiating antituberculous therapy. 1 week later he presented with a recurrence of tuberculosis symptoms and cervical node enlargement. Paradoxical tuberculosis-associated IRIS was diagnosed. Over the next 18 months he presented with several tuberculosis-associated IRIS manifestations that sequentially emerged, despite corticosteroid therapy, then resolved. Photographs show development of massive cervical lymphadenitis (A), a chest wall cold abscess (B, arrows), and a massive right psoas abscess shown here on CT scan (C, arrow) from which over 2 L of pus was aspirated (D). Repeated mycobacterial cultures of aspirates from these collections have been negative. After 6 months on ART his CD4 count was 181 cells per μL and viral load undetectable. After 12 months his CD4 count was 448 cells per μL and viral load 35 copies per mL. This was an unusually prolonged course for paradoxical tuberculosis-associated IRIS given that the median duration of symptoms is reported to be 2 months (see text).

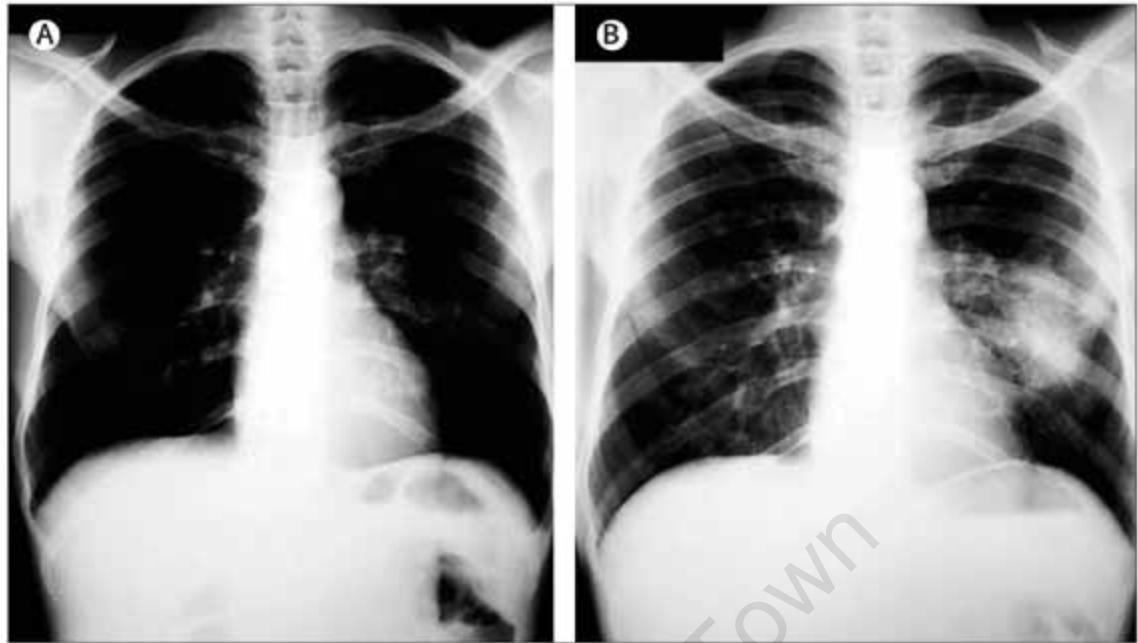


Figure 2. Illustrative case of unmasking tuberculosis-associated IRIS

A 48-year-old HIV-infected man with a CD4 count of 10 cells per μL presented with low-grade fevers, retrosternal chest pain, and a dry cough. Examination was non-contributory. He could not produce sputum and his chest radiograph showed no features of active tuberculosis (A). No other investigations for tuberculosis were available in this resource-limited setting (Uganda). Antiretroviral therapy (ART) was started (zidovudine, lamivudine, and efavirenz). 10 days later he returned acutely unwell with a productive cough. His temperature was 38.7°C and he was in respiratory distress. Chest radiograph now showed left mid-zone consolidation (B) and his sputum was positive for acid-fast bacilli. The unusual rapidity and clinical severity of his tuberculosis presentation was attributed to unmasking tuberculosis-associated IRIS. He responded well to continued ART and tuberculosis treatment.

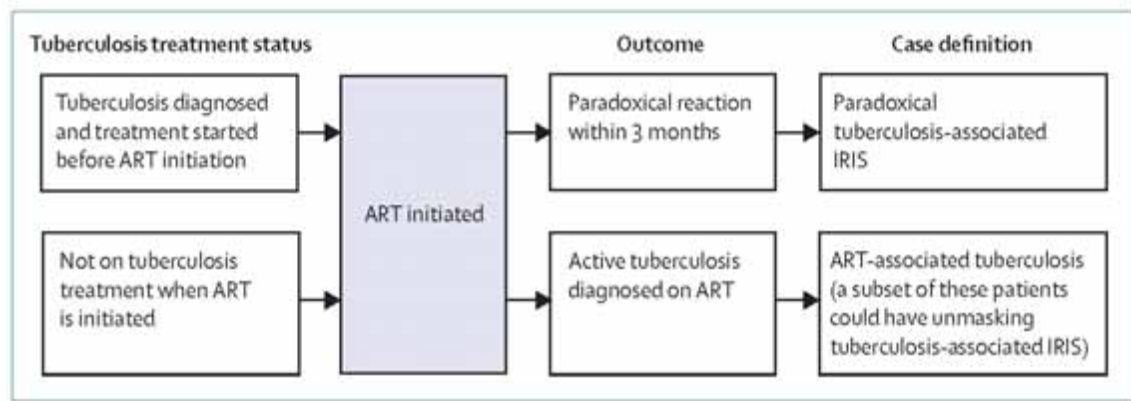


Figure 3. Schematic representation showing the different forms of tuberculosis-associated IRIS and ART-associated tuberculosis
 ART=antiretroviral therapy.

Table

Paradoxical tuberculosis-associated IRIS: cohort studies reported in the literature*

Country	Number of patients on tuberculosis treatment starting ART	Number of patients with paradoxical tuberculosis-associated IRIS	Interval from initiation of ART to IRIS presentation	Risk factors for tuberculosis-associated IRIS
Narita et al (1998) ²²	33	12 (36%)	Mean 15 days (SD 11 days)	Purified protein derivative conversion
Breen et al (2004) ²³	28	8 (29%)	Median 11 days (range 8–18 days)	Starting ART within 6 weeks of tuberculosis diagnosis
Breton et al (2004) ²⁴	37	16 (43%)	Median 12 days (range 2–114 days)	Greater increase in CD4 percentage and CD4/CD8 ratio; disseminated tuberculosis
Kumarasamy et al (2004) ⁸	144	11 (8%)	Median 42 days (range 10–89 days)	..
Shelburne et al (2005) ^{7,21}	86	26 (30%)	Median 46 days (range 3–658 days)	Shorter interval to starting ART; more rapid initial fall in viral load
Michailidis et al (2005) ^{7,25}	28	9 (32%)	Median 0.6 months (IQR 0.1–9.1 months)	Lower baseline CD4 cell count; disseminated tuberculosis; greater CD4 rise on ART
Manosuthi et al (2006) ⁹	167	21 (13%)	Median 32 days (IQR 14–115 days)	Extrapulmonary tuberculosis
Lawn et al (2007) ¹⁰	160	19 (12%)	Median 2 weeks (IQR 1.5–3.5 weeks)	Lower baseline CD4 cell count; shorter interval to starting ART
Burman et al (2007) ²⁶	109	19 (17%)	Median 34 days (IQR 8–97 days)	Black ethnic origin; shorter interval to starting ART; extrapulmonary tuberculosis

..=not reported. ART=antiretroviral therapy. IRIS=immune reconstitution infl ammatory syndrome.

* Only studies where more than eight patients with paradoxical tuberculosis-associated IRIS were reported are included. This table is an updated version of a previously published table.²⁷ Studies are presented in chronological order.

[†]The authors reported 57 cases of tuberculosis, *Mycobacterium avium* complex, and cryptococcal IRIS (26 of 57 were tuberculosis-associated IRIS). Five of these 57 patients started ART before the opportunistic infection was diagnosed, and were thus not paradoxical IRIS cases. The data shown regarding risk factors and median interval relate to all 57 patients.

[‡] 14 tuberculosis-associated IRIS cases were reported. Nine of these were paradoxical tuberculosis-associated IRIS cases. Data shown regarding risk factors relate to all 14 cases.